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Influence of Salt Marsh Vegetation on Carbon Cycling and Microbial Communities

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Influence of Salt Marsh Vegetation on Carbon Cycling and Microbial Communities

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Influence of Salt Marsh Vegetation on Carbon Cycling and Microbial Communities

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TABLE OF CONTENTS

List of Tables

List of Figures

Vegetation zonation drives salt marsh carbon processing and microbial communities

Salt marsh plant-mediated carbon turnover overrides effects of sea-level rise

Vegetation zonation drives salt marsh carbon processing and microbial communities

Abstract

Coastal marshes are important "blue carbon" reservoirs, but it is unclear how vegetation shifts associated with tidal restoration and sea-level rise alter microbial community composition and soil respiration rates. In 2017, we surveyed 20 Connecticut salt marshes (10 unrestricted, 10 tidally restored) and sampled plants and soils from three vegetation zones (*Spartina alterniflora, S. patens, Phragmites australis*). We quantified above- and below-ground biomass, a suite of sediment characteristics (pH, conductivity, soil moisture, organic matter, SO_4^{2-}, Cl^-, and NH_4^+ concentrations), microbial respiration rates (SIR: substrate induced respiration; carbon mineralization), and root-zone bacterial 16S rRNA genes. While only one of our parameters, carbon density, differed between unrestricted and tidally restored marshes, we observed strong differences among vegetation zones. We observed distinct root-zone microbial communities associated with vegetation zones, with sulfate-reducing bacteria being more abundant in *Spartina spp.* zones. Microbial respiration rates (SIR, carbon mineralization) of both *Spartina spp.* zones were higher than *P. australis,* and were positively correlated with both electrical conductivity and belowground biomass. Carbon density was greater in unrestricted marshes than restored sites suggesting that ecosystem services lost during tidal restrictions may take time to rebound. Our findings suggest that salt marsh vegetation can be a useful indicator of hydrologic conditions as well as a predictive tool for estimating microbial respiration rates; however, it is still unclear whether differences in microbial respiration and community composition between vegetation zones is driven by plant or environmental conditions or perhaps an interaction between both.

Introduction

Coastal wetlands are one of the most productive ecosystems on the planet and provide numerous services including storm mitigation (Shepard and others 2014), wildlife habitat (Brawley and others 1998), and provision of a major carbon sink (Barbier and others 2011; McLeod and others 2011). The high productivity of vascular plants in coastal marine ecosystems along with anoxic soils, relatively slow rates of soil decomposition, and negligible methane emissions (Poffenbarger and others 2011), result in the accumulation of deep soil organic matter reservoirs. However, coastal wetlands are increasingly tidally restricted by human-driven coastal development (Nahlik and Fennessy 2016) and threatened by sea-level rise (McLeod and others 2011; Rodríguez and others 2017), both of which can alter plant community composition and the biotic-abiotic feedbacks that underpin carbon cycling and sequestration.

Coastlines around the world are subject to development pressure (Sandi and others 2018), leading to modified tidal regimes that can increase the susceptibility of vegetation to sea-level rise (SLR) (Rodríguez and others 2017). In New England (USA), colonial ditches were created to support marsh haying and grazing activities as well as control mosquitos (Vincent and others 2003; Crain and others 2009). Extensive coastal development resulted in the construction of roads, dikes, and railroads that traverse the majority of northeastern coastal marshes today (Bertness and others 2002; Correll and others 2017). These alterations restrict the exchange of salt water and sediment between marine and coastal ecosystems, reducing flooding frequency and polyhaline conditions, and resulting in plant composition shifts towards more brackish and freshwater species. Restricted marshes are often dominated by the invasive grass *Phragmites australis*, which has a competitive advantage in less saline environments and has deleterious consequences on plant and wildlife diversity (Roman and others 1984; Chambers et al. 2012). Because of less frequent flooding, restricted marshes also experience greater rates of organic

matter oxidation (Portnoy and Giblin 1997). To reduce invasive dominance and subsidence associated with increased oxidation (Burdick and others 1997), restoration efforts in recent decades have focused on reintroducing tidal flooding; impoundment removal and tide-gate installation have led to increased flooding and salinity of numerous coastal wetlands (Burdick and others 1997; Konisky and others 2006). However the effects of tidal restoration on carbon-based services in salt marshes have not been well-documented. While Doroski and others (2019a) found a positive relationship between substrate-induced respiration and time since restoration in brackish wetlands, their design did not account for the potential effects of vegetation.

Strong environmental gradients in salinity and flooding result in distinct zonation in salt marshes, with dominant plants exhibiting differential tolerance to these environmental drivers (Bertness 1991; Mitsch and Gosselink 2007). Under anoxic conditions, toxic, reduced chemicals (e.g., hydrogen sulfide) accumulate in soils, reducing the availability of nutrients to vascular plants (Ponnamperuma 1972). However, vegetation can alter soil conditions by transporting atmospheric oxygen belowground via aerenchymous tissues, as well as exuding low molecular-weight carbon substrates into the rhizosphere. Intensification of tidal flooding due to sea-level rise has induced landward migration of vegetation (Smith 2015; Raposa and others 2017), with low-marsh *Spartina alterniflora* migrating into areas historically dominated by *Spartina patens* (Basso and others 2015). Field and others (2016) quantified the transgression of *S. alterniflora* into high marsh zones of the Long Island Sound, likely because this species is more flood- and salt-tolerant, and oxygenates its rhizosphere more readily than high marsh species (Bertness 1991). On the upper boundary of the marsh platform, dominance of invasive *P. australis* (Basso

and others 2015; Smith 2015) has resulted in high marsh communities, *S. patens* in particular, becoming constricted or squeezed (*sensu* Doody 2004).

While carbon uptake is primarily dictated by the photosynthetic potential of a few graminoid species that dominate the marsh platform, carbon mineralization (i.e., the microbial breakdown of soil organic carbon) is a dominant pathway for carbon emissions. However, the extent to which coastal wetland vegetation and their associated soil microbial communities influence carbon mineralization is largely unknown. Microorganisms are highly sensitive to alterations in their environment and species-specific rhizosphere environments can differ dramatically (Brune and others 2000; Rietl and others 2016), with interactions among vegetation, soil, and hydrology mediating carbon cycling (Gutknecht and others 2006; Moseman-Valtierra and others 2016). Rietl and others (2016) found different bacterial assemblages associated with *S. alterniflora* and *Juncus roemerianus* in Louisiana salt marshes despite little variation in abiotic environments, suggesting differences were primarily driven by plant root exudation. In a New Jersey marsh, Burke and others (2002) found *S. patens* soils to be more diverse in their microbial structure and had greater microbial biomass compared to *P. australis* and suspect that soil flooding may have caused differences in microbial structure beyond the vegetation input. Plant physiological traits may differentially effect carbon cycling. For example, *P. australis* uses the C_3 photosynthetic pathway, which is less water-efficient than the C_4 pathway used by both *S. alterniflora* and *S. patens* (Waller and Lewis 1979). Invasive monotypes of this tall reed are very productive and able to take up large amounts of atmospheric carbon relative to native *Spartina* spp. (Martin and Moseman-Valtierra 2017). However, higher rhizosphere oxidation by lower elevation *Spartina* species may stimulate greater microbial diversity by introducing oxygen for

aerobic respiration and replenish alternative electron acceptors in soils that would otherwise be enriched with anaerobic microorganisms (Emery and Fulweiler 2014).

In order to clarify how tidal restoration and dominant vegetation zones of southern New England salt marshes influence soil carbon cycling and microbial communities, we implemented a 20-site field survey in coastal Connecticut (CT) to address two primary objectives: (1) Compare carbon mineralization and microbial community composition between tidally restored and unrestricted salt marshes. Relative to unrestricted marshes, we expected tidally restored marshes to have greater carbon mineralization rates because of less-saturated soils, and microbial communities to be less diverse because unrestricted marshes are likely to flood and drain more than a recently restored marsh, creating more diverse environmental conditions for bacteria. (2) Investigate how microbial carbon process rates and community composition differs among dominant salt marsh vegetation zones. We predicted that *P. australis,* the highest and driest vegetation zone with abundant aboveground aerenchymous tissue, would have the highest rates of soil carbon mineralization. Due to greater interactions with tidal flooding, we expected lower vegetation zones to have more diverse soil microbial communities.

Methods

Study Sites- We sampled 20 polyhaline salt marshes along the north shore of the Long Island Sound in CT, USA. (Fig. 1). Sites were selected based on their restoration history and abundance of target vegetation: short-form (<30cm tall) *S. alterniflora*, *S. patens* and *P. australis*. We communicated with CT Department of Energy and Environmental Protection (DEEP) staff to identify 10 tidally unrestricted and 10 restored sites (R. Wolfe and H. Yamalis, *personal communication*). Tidally restored sites were historically restricted, but had tidal flow restored via culvert replacement, fill removal, installation of self-regulating tide gates, or tide gate removal

during the last several decades (1970s-2010s). We selected unrestricted sites, those which have not experienced any documented tidal restrictions or subsequent restorations, based on their proximity to tidally restored sites to limit tidal and climate variation.

Field Sampling- At each of the 20 sites, we identified three candidate vegetation zones for sampling based on the following criteria: dominated (>50% relative cover) by the target species, covered an area approximately >35 m^2, and within 100m of the two adjacent vegetation zones upon inspection in the field. Within each vegetation zone, we established three, 1-m^2 plots that were centered in the middle of the zone, perpendicular to the nearest tidal creek, and at least 5m from each other or the zone edge. We sampled all plots within three hours of low tide to control for tidal influence during the peak of the growing season in mid-August 2017. We visually estimated the percent cover (0-100%) of all species in each plot, and every nine plots conducted independent duplicate plots to ensure consistency across sampling teams. Aboveground biomass of a randomly selected 25x25-cm subplot was clipped at the soil surface and composited across the three plots in each zone. We collected three soil cores (5-cm diameter to 10-cm depth; 196 cm^3 volume) from each plot, which were composited by zone and used to estimate bulk density, belowground biomass, and microbial process rates. To characterize microbial communities, we used ethanol-sterilized spoons to subsample ~5g of root-zone soil in the field. Samples collected for microbial analyses targeted soil rather than roots and are considered root-zone soils hereafter. Samples were transferred to Whirl-Pak bags, placed on dry ice during transport to the University of Connecticut Storrs campus, and stored at -80°C until DNA was extracted.

Biomass & Sediment- To separate belowground biomass from the soil matrix, samples were washed over 2-mm sieves. All biomass (above and belowground) was dried at 65℃ for at least 72 hours prior to being weighed. A subsample of belowground biomass from each vegetation

zone was separated into roots and rhizomes to estimate their relative abundance. Dried biomass was pulverized using a ball mill and analyzed for %C and %N content using a Costech ECS 4010 CHNSO Analyzer (Costech Analytical Technologies, Valencia, CA). Bulk density samples were dried at 105°C for at least 48 hours, weighed, pulverized, and similarly analyzed for %C and %N content. A subsample (~5g) of 2-mm sieved, homogenized soil was dried at 105°C for 72 hours to quantify gravimetric soil moisture. We then estimated loss on ignition (LOI) on the subsample by combusting organic matter at 550°C for four hours.

We calculated carbon density by multiplying our bulk density data by % sediment carbon to determine the approximate carbon per given volume.

Soil Wet Chemistry- We used 2-mm sieved, homogenized soils to quantify all wet chemistry parameters. Soil slurries (1:5 ratio of soil to deionized water) were used to determine soil electrical conductivity ($EC_{1:5}$) and $pH_{1:5}$ on 10g of soil. The slurries were well-mixed on a shaker table (160rpms for 10 minutes) then allowed to settle for 15 minutes prior to taking measurements. $EC_{1:5}$ and $pH_{1:5}$ were measured with an Orion Conductivity Cell and an Orion Star A215 pH Conductivity Meter Orion with Ross Ultra pH/ATC Triode at room temperature (25°C). We analyzed water extracts for chloride (Cl^-) and sulfate (SO_4^{2-}) by mixing 2.5g of soil with 25 ml DI water. Samples were shaken at 200rpms for 30 minutes, and centrifuged at 2500 rpms for five minutes. The supernatant was filtered through Whatman GF/F filters. Then samples were run on a Dionex Ion Chromatography System (ICS)-1100 (Thermo Fisher Scientific, Waltham, MA). We extracted soil-water ammonium (NH_4^+-N) and nitrate (NO_3^--N) with 2M KCl (1:10 ratio of soil to KCl), filtered using 110mm Whatman paper (adapted from Keeney and

Nelson 1982), and analyzed extracts on a SmartChem®200 discrete analyzer (Westco Scientific Instruments, Brookfield, CT).

Microbial Respiration Assays- We conducted assays using 2-mm sieved root-zone soils; thus gas accumulation was associated with microbial breakdown of labile carbon substrates rather than root respiration. We used the substrate induced respiration (SIR) method (Anderson and Domsch 1978; West and Sparling 1986) as an index for potential soil microbial activity under amended conditions. Five grams of sieved soil and 10 mL of yeast solution, delivering 20mg yeast/g dry soil, were added to a 40 mL amber vial and sealed with a septum and cap. Headspace CO_2 samples (1 mL) were injected into a LI840A CO_2/H_2O Gas Analyzer (LI-COR, Lincoln, NE) to quantify CO_2 concentrations at time zero, two, and four hours.

Aerobic carbon mineralization rates were measured as carbon dioxide (CO_2) and methane (CH_4) accumulation over a 24-hour period utilizing a Picarro G2201-*i* gas analyzer and two, 16-port distribution manifolds (Johnson 2018). We added ~50g of sieved soil to 196 mL glass canning jars, allowed them to come to room temperature and connected them to the gas analyzer; headspace gas concentrations were measured approximately every two hours over a 24-hour period. We calculated gas flux rate, for both SIR and carbon mineralization, as the linear change in concentration over time, corrected for temperature, atmospheric pressure, and volume, based on the ideal gas law.

Microbial Communities- We processed samples similar to Elmer and others (2017) in which a ~0.5g subsample was aseptically transferred to a power bead tube (MO BIO Power Soil Kit, Carlsbad, CA) and DNA was extracted using the supplied protocols. DNA extractions were verified by gel electrophoresis and DNA was quantified with a NanoDrop spectrophotometer (NanoDrop Lite, Thermo Scientific, Waltham, MA).

The extractions were then shipped to University of Connecticut's MARS (Microbial Analysis, Resources, and Services, Center for Open Research Resources and Equipment, University of Connecticut) for PCR reactions and sequencing. Bacterial 16S rRNA genes were amplified using 30 ng of extracted DNA. The V4 region was amplified using primers 515F and 806R with Illumina adapters and dual indices (8 basepair golay on 3' (Caporaso and others 2012), and 8 basepair on the 5' (Kozich and others 2013)). Samples were amplified in triplicate using GoTaq (Promega) with the addition of 10µg BSA (New England BioLabs). The PCR reaction was incubated at 95˚C for 3.5 minutes, then 30 cycles of 30 s at 95.0°C, 30 s at 50.0°C and 90 s at 72.0°C, followed by final extension as 72.0°C for 10 minutes. PCR products were pooled for quantification and visualization using the QIAxcel DNA Fast Analysis (Qiagen). PCR products were normalized based on the concentration of DNA from 250-400 bp then pooled using the QIAgility liquid handling robot. The pooled PCR products were cleaned using the Mag-Bind RxnPure Plus (Omega Bio-tek) according to the manufacturer's protocol. The cleaned pool was sequenced on the MiSeq platform using v2 2x250 chemistry (Illumina, Inc).

Paired sequences were assembled into contigs using the make.contigs command with default parameters in the mothur software package, only retaining contigs of at least 253 bases. Each contig was further screened to remove any sequences with any ambiguous nucleotide calls or homopolymers of $\underline{\geq}$ 7 bases. Potential chimeric sequences were removed from the dataset with the mothur utilization of vsearch. Sequences were clustered into operational taxonomic units (OTUs) with the OptiClust algorithim in mothur. For analyses of diversity and composition an OTU definition of ≥ 97% sequence identity was used. Taxonomic assignment of sequences was performed with the mothur utilization of classify.seqs against the SILVA 132 ribosomal database (Quast and others 2013)

Statistical Analysis- We tested for normality using Shapiro-Wilk tests, assessed heteroscedasticity by plotting fitted values against residuals, and achieved normality by log transformations when necessary. All response variables were analyzed for restoration and vegetation effects using linear mixed-effects models (*lme4* package) with site as a random factor to account for potential spatial variation. We determined significant effects via analysis of variance (ANOVA) type III on model coefficients at an alpha level of 0.05 with Satterthwaite degrees of freedom (*lmertest* package). When categorical factors were significant, we ran post-hoc pairwise tests of least means squares ($\alpha = 0.05$). Means are presented ± one standard error. All statistical analyses were run in R version 3.5.1 (R Core Team 2017).

To determine which responses best predicted carbon mineralization and SIR rates across vegetation zones, we used corrected Akaike's Information Criterion (AICc) model selection to identify top parsimonious linear mixed-effects models, with site included as a random effect. Results combine microbial respiration rates from restored and unrestricted sites. Soil chemistry ($EC_{1:5}$, soil moisture, and $pH_{1:5}$) and plant biomass (aboveground, belowground) parameters and interactions between soil chemistry and biomass were included in all 13 candidate models. Because of limited sample size, only models with up to 5 parameters (K) were included. Other soil chemistry parameters (SO_4^{2-}, Cl^-, C:N ratios, LOI, %C) were not included in model selection as they were highly correlated (Pearson's correlation > 0.7) with included parameters.

OTU abundance data were uploaded to the phyloseq package for calculation of ordination plots and calculations of alpha diversity. Principal Component Analysis (PCA) was performed on data randomly rarefied to the sample of the smallest sequence dataset (5645 sequences). Non-Metric Multidimensional Scaling (NMDS) was performed when minimal variation was explained by PCA plots. Inter-sample distances were calculated with the Bray-Curtis metric and

PERMANOVA statistics were calculated with the Adonis function in the vegan package. Differentially abundant OTUs were identified using the log_2-fold ratio with the negative binomial generalized linear framework of the DESeq2 software package and post-hoc Wald test (Love *et al.* 2014).

Results

Restoration- Carbon density was greater in unrestricted than tidally restored marshes, but none of the other responses differed between tidally restored and unrestricted marshes (Table 1). However, we observed strong differences in soil chemistry, plant biomass, and microbial carbon processes among vegetation zones.

Soil Chemistry- Our soil chemistry analyses generally reflected the relative elevation and flooding frequency of the three vegetation zones (Table 2). Soil $EC_{1:5}$ was highest in *S. alterniflora* zones (7.6 $\pm$ 0.5 mS cm^{-1}) and lowest in *P. australis* zones (4.7 $\pm$ 0.4 mS cm^{-1}). Salt ions (SO_4^{2-}, Cl^-) followed a similar pattern across vegetation zones, with sulfate ($R^2 = 0.62$) and chloride ($R^2 = 0.76$) concentrations positively correlated with $EC_{1:5}$. The lower salt concentration in *P. australis*-dominated zones is most likely due to less frequent flooding and lower % soil moisture (Table 2). Only 12% of NO_3^- were above detection limit (0.18ppm) and were not included in the model selection.. 77% of NH_4^+ samples were above detection limit (0.33ppm), but we did not detect differences among vegetation zones.

Biomass, C, and N Content - Aboveground biomass differed among vegetation zones ($F_{2, 22.9} = 6.7$, $p < 0.01$), with greater biomass in *P. australis* than *Spartina* spp. zones. However, this trend was reversed for belowground biomass ($F_{2, 35.9} = 64.9$, $p < 0.001$), with *Spartina* spp. zones having greater belowground biomass than *P. australis* (Table 2). *Spartina* spp. also allocated a

higher percentage of belowground biomass to roots (60%) than *P. australis*, which had equal parts roots and rhizomes (Table 2). Aboveground %C and %N were greater in *P. australis* zones than the native *Spartina* spp.-dominated zones ($F_{2, 56} = 18.74$, $p < 0.001$; $F_{2, 38} = 9.99$, $p < 0.001$, respectively). Belowground %C was greater in the *Spartina* spp. zones ($F_{2, 36.9} = 4.21$, $p < 0.05$), but %N did not differ among vegetation zones ($F_{2, 36.8} = 0.43$, $p > 0.05$). %C in the soil sediment was greater in the *Spartina* spp. zones ($F_{2, 38} = 9.67$, $p < 0.001$); however C:N ratios were higher in *P. australis* than *Spartina* spp. zones ($F_{2,56} = 20.09$, $p < 0.001$). Carbon density did not differ among vegetation zones, however it was greater in unrestricted sites ($F_{1, 44} = 5.35$, $p < 0.05$).

Microbial Response- We observed different carbon mineralization and SIR rates (CO_2 accumulation) among vegetation zones ($F_{2, 37.5} = 38.2$, $p < 0.001$; $F_{2, 56} = 17.3$, $p < 0.001$, respectively). Methane accumulation rates were low and not included in carbon mineralization estimates as over half of the samples were below detection limit (0.012 ppm CH_4 hr^{-1}), likely due to sulfate reduction being more thermodynamically favorable in sulfur-rich salt marshes. For both carbon mineralization and SIR, we observed similar patterns among vegetation zones, with greater microbial respiration in *S. alterniflora* zones and the lowest rates in *P. australis* zones (Table 2; Fig. 2). There was a positive correlation between carbon mineralization and SIR ($R^2 = 0.40$), but carbon mineralization rates were an order of magnitude lower than SIR results most likely because of the additional carbon, nitrogen, and phosphorus contributed by yeast added during SIR assays;; untransformed data for carbon mineralization and SIR rates, respectively, were *S. alterniflora*: 7.9 $\pm$ 0.9 µmol CO_2 g C^{-1} hr^{-1} vs. 98.4 $\pm$ 0.2 µmol CO_2 g C^{-1} hr^{-1}, *S. patens*: 5.6 $\pm$ 0.7 µmol CO_2 g C^{-1} hr^{-1} vs. 70.0 $\pm$ 6.6 µmol CO_2 g C^{-1} hr^{-1}, and *P. australis*: 2.2 $\pm$ 0.4 µmol CO_2 g C^{-1} hr^{-1} vs. 38.6 $\pm$ 4.9 µmol CO_2 g C^{-1} hr^{-1}.

Our model selection results suggest that belowground biomass and salinity were the best predictors for observed differences in microbial respiration. Carbon mineralization rates were largely predicted by positive relationships with belowground biomass and $EC_{1:5}$ (Table 3; Fig. 3), with the top models explaining 58% and 62% of the variation. SIR was also positively associated with belowground biomass and $EC_{1:5}$ (Table 4), however the top models only explained 35% and 31% of the variation.

Microbial Communities- Rarified sequences were used to create a non-metric multidimensional scaling plot (Fig. 4). The three vegetation zones had significantly different centroids of their respective ellipses (Adonis test p-value < 0.001). However, there was considerable overlap between microbial communities from the three vegetation zones (beta dispersion p value < 0.001) indicating that the ellipses were not distinct. We did not observe a significant difference in inverse Simpson index among vegetation zones ($F_{2, 57} = 2.88$, $p = 0.06$).

Spartina spp. zones had more similar sediment microbial communities than *P. australis*-dominated zones (Fig. 4). Our data suggest that particular OTUs were more abundant in certain vegetation zones, with differentially abundant OTUs belonging largely to five phyla with a large proportion representing *Proteobacteria* and *Bacteroidetes* (Fig. 5). We observed 266 OTUs that differed in abundance between vegetation zones as identified by likelihood ratio tests (Fig. 5). Using Wald tests, we determined that there were 15 differentially abundant OTUs between *S. alterniflora*- and *S. patens* zones, 88 between *S. patens*- and *P. australis* zones, and 246 between *S. alterniflora*- and *P. australis* zones.

Discussion

We conducted a 20-site field survey along coastal CT to investigate the role of tidal restoration and vegetation on microbial respiration rates and community structure. Our prediction of tidal restoration affecting microbial respiration was not supported as we did not observe differences between tidally restored and unrestricted marshes within vegetation zones on any responses we measured except for carbon density. We had anticipated that soils dominated by *P. australis* would have the greatest rate of carbon mineralization; however, we found that carbon mineralization rates were positively correlated with belowground biomass and salinity, with the highest rates in *S. alterniflora* zones and lowest in zones dominated by *P. australis*. While we found high dispersion in our microbial community structures, there was greater abundance of unique OTUs in lower marsh zones.

Effects of tidal restoration- Tidally restricted marshes are ubiquitous along developed coastlines, resulting in a suite of deleterious consequences including surface elevation subsidence and reduced salinity levels that promote brackish vegetation such as invasive *P. australis* (Warren and others 2002). Reconnection of tidal flows by removing tide gates and enlarging culverts, restores the exchange of salt water and sediments between restricted marshes and estuaries allowing natural flooding regimes and salinity to rebound (Konisky and others 2006). Over the past several decades, the state of CT has initiated >80 tidal restorations with over 730 hectares of tidal marsh restored, resulting in a 12% increase in total acres of brackish and salt marshes in the state (Rozsa 2012); the unrestricted sites we sampled ranged in time since restoration from five years to four decades (Roger Wolfe, *personal communication*).

Surprisingly, we did not observe differences between tidally restored and unrestricted marshes within similar vegetation zones in microbial respiration rates, nor for any of our soil chemistry responses, but we did find carbon density to be higher in unrestricted marshes. Perhaps

carbon density takes longer to respond to restoration actions than several decades or even centuries and plants take time to recolonize and accumulate biomass (Ballantine and Schneider 2009). Carbon density was also slightly greater compared than previous studies perhaps because of our limited sampling depth. Previous salt marsh reviews, such as Chmura and others (2003) and CEC (2015) examined carbon density to depths of 50cm and 20cm respectively; both observed a decline in carbon density with depth. Our findings suggest that surface soil (0-10cm) microbial respiration responds relatively quickly to tidal restoration and had analogous rates as reference unrestricted marshes. We did not find correlations between time since restoration and carbon mineralization ($R^2 = 0.01$) or SIR ($R^2 = 0.01$). When we examined time since restoration for soil respiration among each vegetation zone separately restoration, there were no significant correlations found. We collected sediment cores to a depth of 10 cm, anticipating this region to be the most microbially-active, however soil organic matter can take decades to respond to restoration management (Craft and others 1999; Warren and others 2002). In brackish marshes, Doroski and others (2019a) observed higher potential denitrification rates and SIR rates with time since restoration, but not for carbon mineralization. Across vegetation zones, we did not observe differences in soil chemistry responses ($EC_{1:5}$, $pH_{1:5}$, Cl^-, SO_4^{2-}, NH_4^+, LOI, soil moisture) between tidally restored and unrestricted marshes. This is likely because salt marsh vegetation zonation is highly constrained by the ability of the dominant species to withstand flooding and salinity stressors, and thus vegetation can be considered a biological indicator of hydrologic and physio-chemical conditions (Smith and Warren 2012).

Vegetation Zones Differ- Our field survey revealed strong differences among vegetation zones in plant biomass, soil chemistry, and microbial process rates, highlighting the interconnected nature of structure and function in salt marshes and the importance of tidal flooding in regulating

vegetation zonation (Warren and others 2002). Our findings suggest that vegetation may be a useful indicator of key carbon- and nitrogen-based processes in salt marshes as vegetation zones had distinct microbial respiration rates (current study) and denitrification potentials (Ooi and others *in prep*). Because tidal restoration and SLR change the composition and areal extent of salt marsh vegetation (Smith and Warren 2012), remotely sensed coastal vegetation data sets (e.g., Correll and others 2018) could be used to scale empirical estimates of carbon- and nitrogen-based processes to regional scales (Ooi and others *in prep*).

High allocation to belowground biomass and the ability to oxygenate soils allow low marsh vegetation (i.e., *S. alterniflora*) to tolerate frequent tidal inundation (Bertness 1991); our data support this as we observed higher belowground biomass, soil salinity ($EC_{1:5}$, Cl^-, SO_4^{2+}), and soil moisture in lower vegetation zones (*S. alterniflora* and *S. patens*) that are more frequently inundated than higher elevation *P. australis*-dominated zones. Microbial responses and communities also followed this a pattern of higher respiration rates and greater abundance in lower elevated zones (*Spartina* spp.) than the higher elevated zones (*P. australis*). In contrast to our predictions, carbon mineralization and SIR rates were highest in soils dominated by *S. alterniflora* and lowest in soils dominated by *P. australis*; we had predicted soils from *P. australis*-dominated zones would have the greatest carbon mineralization rates because they are relatively dry and have abundant aboveground aerenchymous biomass that could stimulate microbial activity. However, our top models consistently included belowground biomass and $EC_{1:5}$, which were positively correlated with microbial respiration rates, suggesting that carbon mineralization was best predicted and perhaps driven by both biotic (belowground inputs) and abiotic factors (salinity).

Plant inputs such as oxygenation of the soil and carbon exudates are known to stimulate microbial activity (Rietl and others 2016). However it is less apparent how increased salinity may influence carbon mineralization rates. Increased salinity in soils may promote greater sulfate-reduction, but salinity coupled with more frequent inundation promotes higher concentrations of hydrogen sulfide and chloride, both of which may cause inhibition of plant nutrient uptake and ionic stress (Luo and others 2019); which may reduce interactions between vegetation and microbial respiration rates. Nevertheless the response of soils across vegetation zones was similar for both carbon mineralization and SIR. Perhaps the breakdown of organic carbon in wetlands is not explicitly driven by a salinity gradient but another closely allied factor, such as soil moisture or oxygen availability. Our carbon mineralization rates were conducted under aerobic conditions whereas the soils of *S. alterniflora* are often anoxic due to tidal inundation. Exposing anoxic soils to oxic environments may heighten carbon mineralization rates as anaerobes are unable to utilize complex organic carbon substances, but have the high potential to rapidly recycle labile carbon (Kristensen and others 2008). The addition of yeast for SIR is more than simply carbon fertilization as yeast also contains other components such as nitrogen and phosphorus that may have influenced our observed rates of respiration. While laboratory assays do not exactly replicate field conditions, differential carbon respiration rates across vegetation zones was apparent.

Plant inputs may play a large role in microbial respiration. We examined different vegetation zones across a salt marsh gradient and our results suggest that plants, via priming, influence microbial communities and microbial respiration. Low marsh plants, such as *S. alterniflora*, tend to have increased allocation of belowground biomass beneath the soil surface to provide structural support to limit erosion as well as greater surface area for root oxidation

(Bertness 1991). We observed less belowground biomass in *P. australis*-dominated surface soils, which may have limited the plant mediated interaction with microbial respiration. However it has been documented that the belowground biomass of *P. australis* extends deeper into the soil than native marsh species (Mozdzer and others 2016). In addition, nutrients and organic material concentrations are known to change with depth (Craft and others 1999). We sampled the top 10-cm to examine the most microbially-active soils, but we are aware that vegetation and nutrients differ with depth. Low molecular weight carbon exudates are readily metabolized by microbial communities (Farrar and others 2003) whereas *P. australis* tissues contains relatively higher levels of phenol-rich, recalcitrant carbon compounds that are less readily utilized by microorganisms (Kim and others 2018). Oxygenation of soils also play a role in increasing soil microbial respiration (Sutton-Grier and Megonigal 2011; Chapman and others 2019). As conditions become more oxic, aerobic microorganisms can rapidly metabolize carbon compounds. Howes (1994) found that *S. alterniflora* releases a significant portion of oxygen into the rhizosphere via aerenchymous tissue. However, plant characteristics such as biomass are highly correlated with soil chemistry parameters thus it is difficult to disentangle whether differences in microbial respiration rates are driven by biotic factors such as root biomass and root exudates or by abiotic factors such as flooding frequency and increased salinity. Future studies should investigate the independent and interactive effects of biotic (plant inputs) and abiotic (salinity and flooding) to elucidate their role on salt marsh microbial respiration rates.

Microbial Communities- While vegetation has been identified as an important driver of microbial composition in terrestrial settings (Grayston and others 1998; Ladygina and Hedlund 2010), it has been less studied in coastal wetlands (Rietl and others 2016) possibly due to the numerous environmental factors (i.e., soil salinity, sulfide concentrations, redox potential) that can vary

among wetland plants (Bertness 1991). Our study helps bridge this gap as we systematically characterized microbial communities across dominant salt marsh vegetation zones in 20 marshes, on the north shore of Long Island Sound. Whereas previous studies typically examine one to a few sites (Ravit and others 2003; Elmer and others 2017; Rietl and others 2016). We anticipated high variation across marshes because community structure is often site specific (Ravit and others 2007; Bowen and others 2009; Simon and others 2017), but expected to observe trends along environmental gradients. Although we did not observe strong differences in community composition among vegetation zones, certain bacteria and bacterial groups were differentially abundant. For example, within *Spartina* spp.-dominated soils, we found higher proportions of the sulfur-reducing delta-Proteobacteria, (Kearns and others 2016), suggesting that the physio-chemical environment may drive both vegetation zonation and microbial communities in coastal wetlands (Chrzanowski and Spurrier 1987). *Spartina* spp. zones were also associated with greater abundance of the phylum Bacteroidetes, bacteria associated with plant roots (Gkarmiri and others 2017) and the metabolism of recalcitrant carbon (Fierer and others 2007; Ai and others 2015). Given that we observed higher rates of carbon mineralization in *Spartina* spp.-dominated zones, the higher abundance of these two bacteria groups suggests that there may be feedbacks between vegetation, microbial community structure, and microbial process rates. While we observed higher abundance of bacteria that metabolize plant-derived carbon in vegetation zones with higher microbial respiration rates, sequencing-based microbial abundance estimates are not necessarily indicative of microbial activity, as active and non-active sequences are not differentiated with this approach (Blazewicz and others 2013). For future studies, it would be valuable to quantify carbon cycling, microbial community structure, as well as examine extracellular enzymes associated with microbial metabolism and nutrient cycling. By

examining extracellular enzymes such as *beta*-glucosidase and phenol oxidase we may gain a better understanding of which bacteria are actively metabolizing plant-derived compounds (Freeman and others 2001; Sinsabaugh 2010; Morrissey and others 2014).

In general, root-zone soils dominated by *Spartina* spp. had more similar soil characteristics than *P. australis* (Table 2), a pattern consistent with our observations of in the soil-root zone microbial communities. While there was considerable overlap among microbial communities (Fig. 4), the two *Spartina*- dominated zones had more in common in bacterial structure than with *P. australis*-dominated soils. *Spartina alterniflora* and *S. patens* shared relatively similar microbial communities with only 15 OTUs being differentially abundant between the two vegetation zones; whereas the greatest number of differentially abundant OTUs were between *S. alterniflora* and *P. australis* with 246 (Fig. 5). These OTUs generally represented delta-Proteobacteria and Bacteroidetes with more present in the *Spartina*-dominated zones. However, at least two of the most differentially abundant OTUs found in *P. australis*-dominated zones were nitrogen fixing bacteria; while we observed no differences in NO_3^- nor NH_4^+, Ooi and others (*in prep*) observed greatest denitrification potentials in these same *P. australis* zones, suggesting rapid utilization of limited nitrogen by the microbial community.

Management Implications- As coastal marshes are squeezed by both sea-level rise and coastal development (Doody 2004), coastal managers will increasingly need to make challenging decisions about potential tradeoffs among biodiversity, vegetation structure, and ecosystem function. Our findings suggest that vegetation can be both an indicator of salt marsh hydrologic conditions as well as a useful predictive tool for estimating microbial respiration rates. As rising sea-levels continue to shift marsh vegetation composition, linking vegetation zones with carbon mineralization rates may aid land management decisions in terms of where to prioritize

conservation efforts and restoration activities. Shifting coastal wetland vegetation may trigger a cascade of effects altering carbon cycling and storage capacity. As plant zones migrate inland, there may be increased soil carbon respiration as more frequent flooding promotes species with abundant belowground biomass (i.e., *S. alterniflora*), elevated salinity increases sulfate reduction, and senesced plant tissues increase the availability of labile carbon (Rooth and others 2003; Chambers and others 2011). Coupling our estimates of aboveground biomass (an index of carbon uptake (Morris 2007; Byrd and others 2018)) and carbon mineralization rates, our data suggest that *P. australis* may promote carbon storage.

The purpose of many tidal restorations in mid-Atlantic and northeastern U.S. marshes has been to reduce the abundance of invasive *P. australis* (Chambers and others 2012) because this dominant macrophyte reduces habitat quality for a range of species (Roman and others 1984; Chambers and others 1999). However, prioritizing carbon sequestration is becoming more common during ecological restoration (Bullock and others 2011; McLeod and others 2011). In an era of limited conservation funds, tailoring management objectives to achieve realistic outcomes requires an understanding of the potential tradeoffs associated with vegetation management (i.e., biodiversity vs. carbon sequestration and storage). Our findings reinforce those of others (Windham and Lathrop 1999; Moseman-Valtierra 2016) that have observed *P. australis* to store soil carbon more effectively than both *Spartina* spp. Thus, prioritizing biodiversity objectives and allocating financial resources to focal marshes most resistant to invasion and valuing carbon-based and resilience services (erosion, sediment accumulation) provided by *P. australis*- dominated marshes may be a more practical management strategy than eradication efforts.

Conclusions- We conducted a 20-site coastal marsh survey to investigate how tidal restoration and vegetation zonation affect microbial respiration and community structure in surface soils. We found no difference between tidally restored and unrestricted marshes in soil chemistry, plant biomass, soil carbon respiration rates, or microbial communities, indicating that restoration efforts over the past 40 years in CT have been able to restore carbon processing and microbial communities. However, since our study suggests that vegetation could be utilized as an indicator of soil carbon processing in southern New England coastal marshes, we recommend scaling based on vegetation zones to more holistically quantify the effects of restoration on carbon processing. Soils associated with *P. australis* had lower rates of carbon mineralization and higher aboveground biomass than *Spartina* spp. zones, suggesting greater potential for carbon sequestration. While we found that soil salinity and belowground biomass are important predictors of microbial respiration rates and microbial communities, flooding frequency, soil properties, and vegetation are inherently confounded during field surveys. Therefore experimental tests isolating the effects of coastal flooding and vegetation are necessary to further our understanding of the relative roles of abiotic and biotic controls on coastal marsh carbon cycling.

Table 1. ANOVA results of linear mixed-effects models with site as a random factor evaluating the effects of restoration status and vegetation zone on soil chemistry, C, N content, biomass, and microbial response. There was no effect of restoration for the majority of variables except for carbon density. (NS: $p > 0.05$, *: $p < 0.05$, **: $p < 0.001$, ***: $p < 0.001$)

	Variable	Factor	*df*	F-value	p-value
Soil Chemistry	**$EC_{1:5}$ (mS cm^{-1})**	Restoration	1	0.34	NS
		Vegetation	2	19.71	***
	Chloride (mg kg Dry $Soil^{-1}$)	Restoration	1	0.16	NS
		Vegetation	2	23.34	***
	Sulfate (mg kg Dry $Soil^{-1}$)	Restoration	1	0.03	NS
		Vegetation	2	16.99	***
	$pH_{1:5}$	Restoration	1	0.002	NS
		Vegetation	2	0.5	NS
	% Soil Moisture	Restoration	1	0.11	NS
		Vegetation	2	17.21	***
	LOI	Restoration	1	0.03	NS
		Vegetation	2	8.52	***
	NH_4^+ (mg NH_4-N kg Dry $Soil^{-1}$)	Restoration	1	2.33	NS
		Vegetation	2	1.52	NS
C, N Content	**Sediment %C**	Restoration	1	0.0002	NS
		Vegetation	2	9.66	***
	Sediment %N	Restoration	1	0.003	NS
		Vegetation	2	1.97	NS
	Sediment C:N	Restoration	1	0.005	NS
		Vegetation	2	20.09	***
	Aboveground Biomass %C	Restoration	1	0.43	NS
		Vegetation	2	10	***
	Aboveground Biomass %N	Restoration	1	0.13	NS
		Vegetation	2	14.08	***
	Aboveground C:N	Restoration	1	0.002	NS
		Vegetation	2	23.89	***
	Belowground Biomass %C	Restoration	1	0.09	NS
		Vegetation	2	4.22	*
	Belowground Biomass %N	Restoration	1	0	NS
		Vegetation	2	0.43	NS
	Belowground C:N	Restoration	1	0.008	NS
		Vegetation	2	4.25	*
	Carbon Density	Restoration	1	5.35	*
		Vegetation	2	2.15	NS
Biomass	**Aboveground Biomass (kg m^{-2})**	Restoration	1	1.33	NS
		Vegetation	2	6.73	**
	Belowground Biomass (kg m^{-2})	Restoration	1	0.04	NS
		Vegetation	2	64.79	***
Microbial Response	**log Carbon Mineralization (µmol CO_2 gC^{-1} hr^{-1})**	Restoration	1	0.32	NS
		Vegetation	2	38.24	***
	log SIR (µmol CO_2 gC^{-1} hr^{-1})	Restoration	1	0.01	NS
		Vegetation	2	17.29	***

Table 2. Results of linear mixed-effects models with site as a random factor testing the effects of restoration status and vegetation zone on soil chemistry, C,N content, biomass, and microbial response. Vegetation zones that do not share the same letter were different from one another.

		S. alterniflora	*S. patens*	*P. australis*
		Fixed Effects Estimate (SE)	Fixed Effects Estimate (SE)	Fixed Effects Estimate (SE)
Soil Chemistry	**$EC_{1:5}$ (mS cm^{-1})**	7.6 (0.5)^{a}	6.8 (0.5)^{a}	4.7 (0.4)^{b}
	log Chloride (mg kg Dry $Soil^{-1}$)	4.31 (0.25)^{a}	3.91 (0.24)^{1}	2.7 (0.26)^{b}
	Sulfate (mg kg Dry $Soil^{-1}$)	12.33 (1.4)^{a}	9.49 (1.4)^{a}	4.33 (1.4)^{b}
	$pH_{1:5}$	6.54 (0.17)^{a}	6.70 (0.17)^{a}	6.66 (0.14)^{a}
	LOI	36.4 (3.3)^{a}	38.0 (3.3)^{a}	25.1 (5.3)^{b}
	% Soil Moisture	81.8 (4.0)^{a}	79.31 (4.0)^{a}	60.2 (4.8)^{b}
	NH_4^+ (mg NH_4-N kg Dry $Soil^{-1}$)	3.05 (0.6)^{a}	4.09 (0.6)^{a}	3.55 (0.5)^{a}
C, N Content	**Sediment %C**	24.4 (1.9)^{a}	22.7 (1.9)^{a}	16.3 (2.7)^{b}
	Sediment %N	1.18 (1.0)^{a}	1.08 (1.0)^{a}	0.98 (0.15)^{a}
	Sediment C:N	21.56 (0.81)^{a}	21.07 (0.81)^{a}	16.86 (0.67)^{b}
	Aboveground Biomass %C	40.22 (1.03)^{a}	43.07 (1.03)^{b}	44.79 (0.85)^{b}
	Aboveground Biomass %N	1.03 (0.08)^{a}	0.81 (0.08)^{b}	1.22 (0.6)^{c}
	Aboveground Biomass C:N	40.43 (2.98)^{a}	57.51 (2.98)^{b}	39.00 (2.43)^{a}
	Belowground Biomass %C	44.03 (2.2)^{a}	44.93 (2.1)^{a}	39.17 (1.8)^{b}
	Belowground Biomass %N	0.93 (0.07)^{a}	0.93 (0.07)^{a}	0.94 (0.06)^{a}
	Belowground Biomass C:N	51.82(3.24)^{a}	52.05 (3.15)^{a}	43.88 (2.57)^{b}
	Carbon Density	0.12 (0.02) ^{a}	0.11 (0.02) ^{ab}	0.081 (0.02) ^{b}
Biomass	**Aboveground Biomass (kg m^{-2})**	1.36 (0.45)^{a}	1.52 (0.45)^{a}	2.87 (0.38)^{b}
	Belowground Biomass (kg m^{-2})	13.3 (0.7)^{a}	10.3 (0.7)^{b}	5.1 (0.6)^{c}
Microbial Response	**log Carbon Mineralization (µmol gC^{-1} hr^{-1})**	2.00 (0.16)^{a}	1.58 (0.15)^{b}	0.67 (0.16)^{c}
	log SIR (µmol gC^{-1} hr^{-1})	4.5 (0.17)^{a}	4.1 (0.17)^{b}	3.5 (0.14)^{c}

Table 3. Candidate multiple regression models for carbon mineralization rates for each possible number of model coefficients (K), including intercept and random site effect. Reported statistics include Akaike's Information Criterion (AIC), adjusted AIC (AIC_c), the difference between candidate and top model AICc (ΔAICc), the weight of the model, and the conditional R^2.

Model	**K**	**AICc**	**ΔAICc**	**Weight**	**Conditional R^2**
Belowground	3	114.5896	0	0.83	0.58
$EC_{1:5}$ + Belowground	4	118.7058	4.1162	0.11	0.62
$EC_{1:5}$ + $pH_{1:5}$	4	120.4811	5.891539	0.04	0.61
$EC_{1:5}$	3	122.065	7.475491	0.02	0.56
$EC_{1:5}$ + Aboveground	4	127.4935	12.903917	0.00	0.55
Soil Moisture + Belowground	4	129.5203	14.930772	0.00	0.59
Aboveground	3	134.0363	19.446702	0.00	0.12
$EC_{1:5}$ + Soil Moisture + Belowground	5	135.3651	20.775515	0.00	0.63
$pH_{1:5}$	3	135.9031	21.313536	0.00	0.26
$EC_{1:5}$ + Soil Moisture	4	136.2025	21.612965	0.00	0.64
$EC_{1:5}$ + Soil Moisture + Aboveground	5	141.7691	27.179535	0.00	0.63
Soil Moisture	3	142.7045	28.114942	0.00	0.38
Soil Moisture + Aboveground	4	143.0884	28.498836	0.00	0.47

Table 4. SIR AIC table. Candidate multiple regression models for carbon mineralization rates for each possible number of model coefficients (K), including the intercept and the random effect of site. Reported statistics include Akaike's Information Criterion, adjusted Akaike's Information Criterion, the difference between the candidate and best model's AICc (Δ AICc), the weight of the model and the conditional R^2.

Model	**K**	**AICc**	**ΔAICc**	**Weight**	**Conditional R^2**
Belowground	3	98.78608	0	0.57	0.35
$EC_{1:5}$	3	100.492	1.70592	0.24	0.31
$EC_{1:5}$ + Belowground	4	101.78845	3.002365	0.13	0.39
$EC_{1:5}$ + $pH_{1:5}$	4	104.00338	5.217297	0.04	0.31
$EC_{1:5}$ + Aboveground	4	106.29896	7.51288	0.01	0.3
Soil Moisture + Belowground	4	107.76861	8.982533	0.01	0.43
$EC_{1:5}$ + Soil Moisture	4	112.39801	13.61193	0.00	0.37
Soil Moisture	3	114.14797	15.36189	0.00	0.26
Aboveground	3	114.25668	15.470598	0.00	0.08
$EC_{1:5}$ + Soil Moisture + Belowground	5	114.51651	15.730425	0.00	0.44
$pH_{1:5}$	3	116.84741	18.061332	0.00	0
Soil Moisture + Aboveground	4	117.21722	18.43114	0.00	0.28
$EC_{1:5}$ + Soil Moisture + Aboveground	5	118.40793	19.621847	0.00	0.37

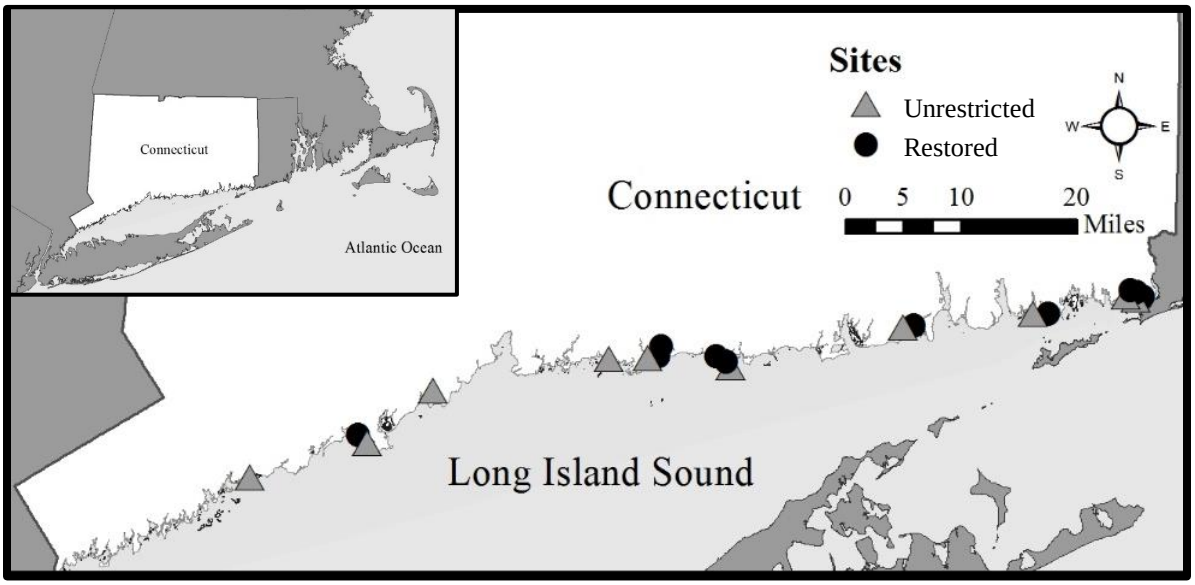

Figure 1. Location of 20 tidal marshes along CT coastline sampled during our summer 2017 field survey; ten tidally-restored (black circles) and ten unrestricted (grey triangles) sites were sampled. Within each site samples were collected from the three dominant vegetation zones: *Spartina alterniflora*, *Spartina patens*, and *Phragmites australis*. All samples were collected within three hours of low tide. The location of the sites were in attempt to represent the span of the Connecticut coastline; our western most site was located in Westport, CT and our eastern most site was located in Stonington, CT.

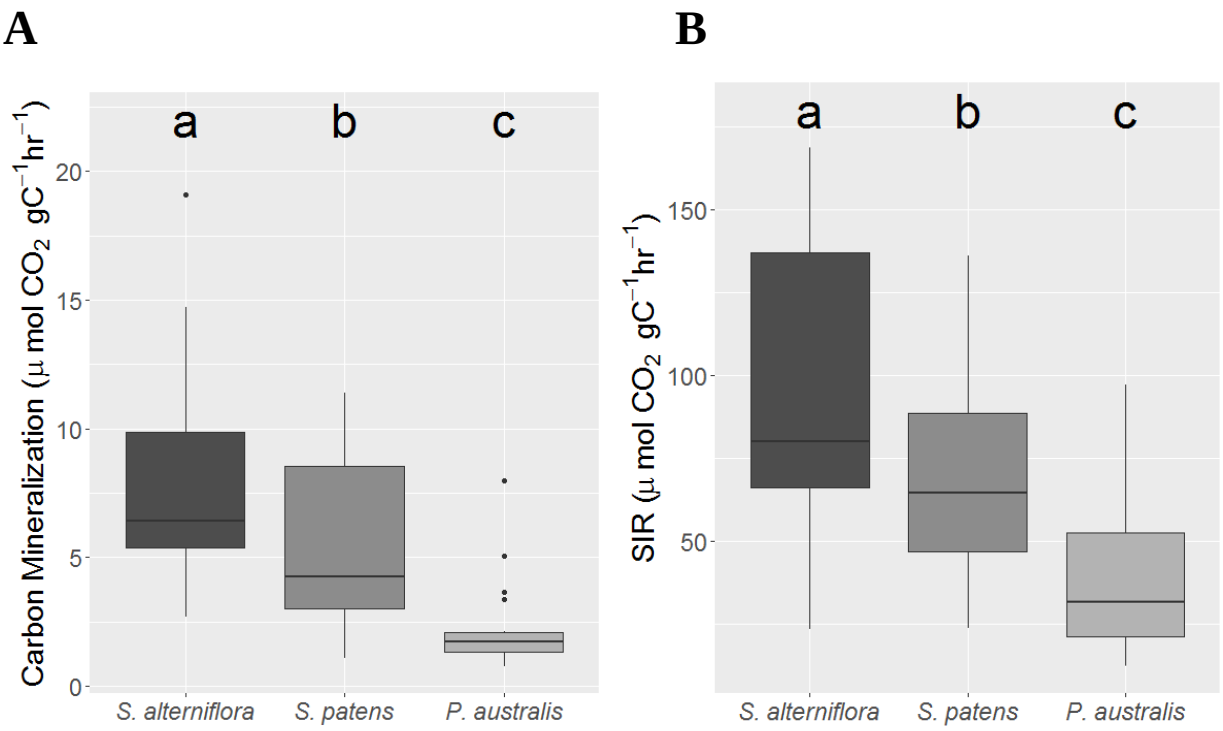

Figure 2. Boxplots of laboratory assays of untransformed (A) Carbon mineralization and (B) Substrate-Induced Respiration (SIR) for soils collected from three dominant vegetation zones: *Spartina alterniflora*, *Spartina patens*, and *Phragmites australis* (n = 20 sites) across coastal marshes in CT. SIR had rates a magnitude higher than carbon mineralization due to the addition of yeast. Both responses demonstrate a similar pattern with greatest mineralization rates found in *S. alterniflora* zones and the least from *P. australis*. Letters represent differences among vegetation zones from mixed-effects model statistical analysis for each response.

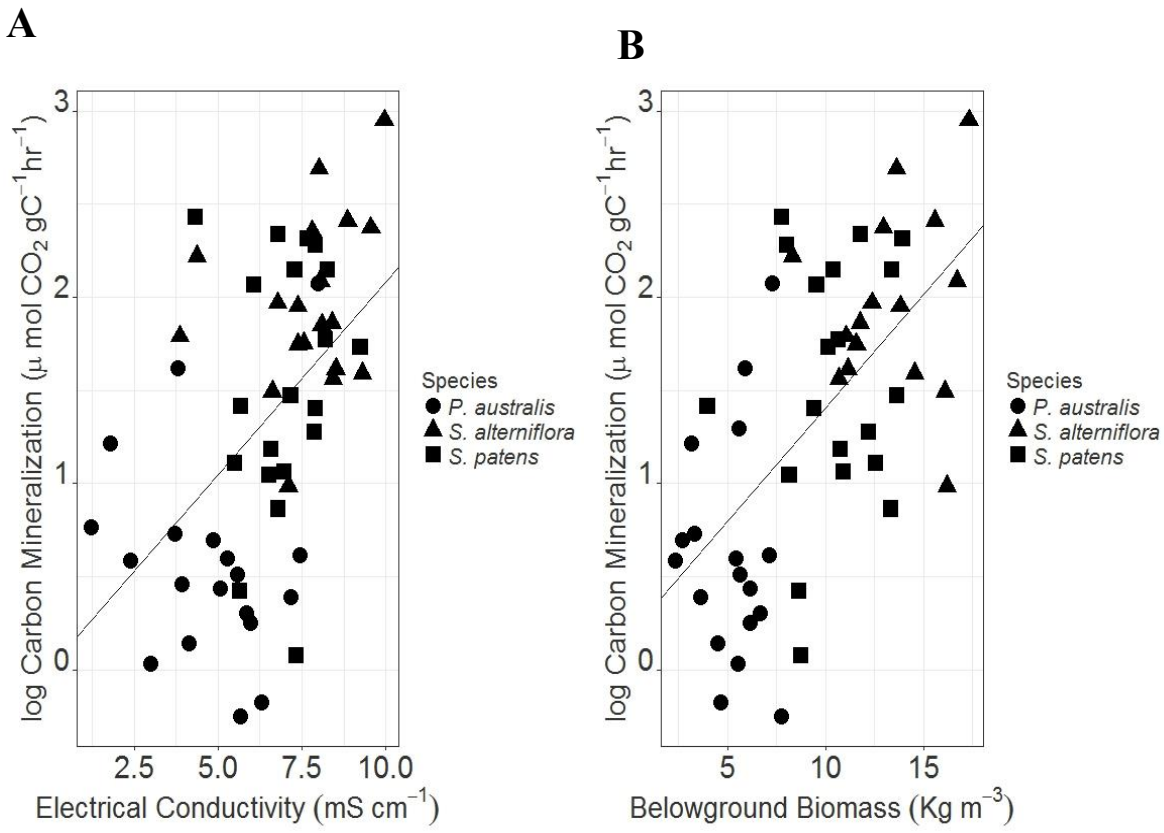

Figure 3. Regression lines of log-transformed carbon mineralization against (A) $EC_{1:5}$ (R^2 = 0.25) and (B) belowground biomass (0-10cm) (R^2 = 0.39). Both explanatory variables were in the top models predicting carbon mineralization rates and were positively correlated; as $EC_{1:5}$ or belowground increased, carbon mineralization increased. However the random effect, site, added to their respective R^2 values which suggests that location also influences carbon mineralization rates.

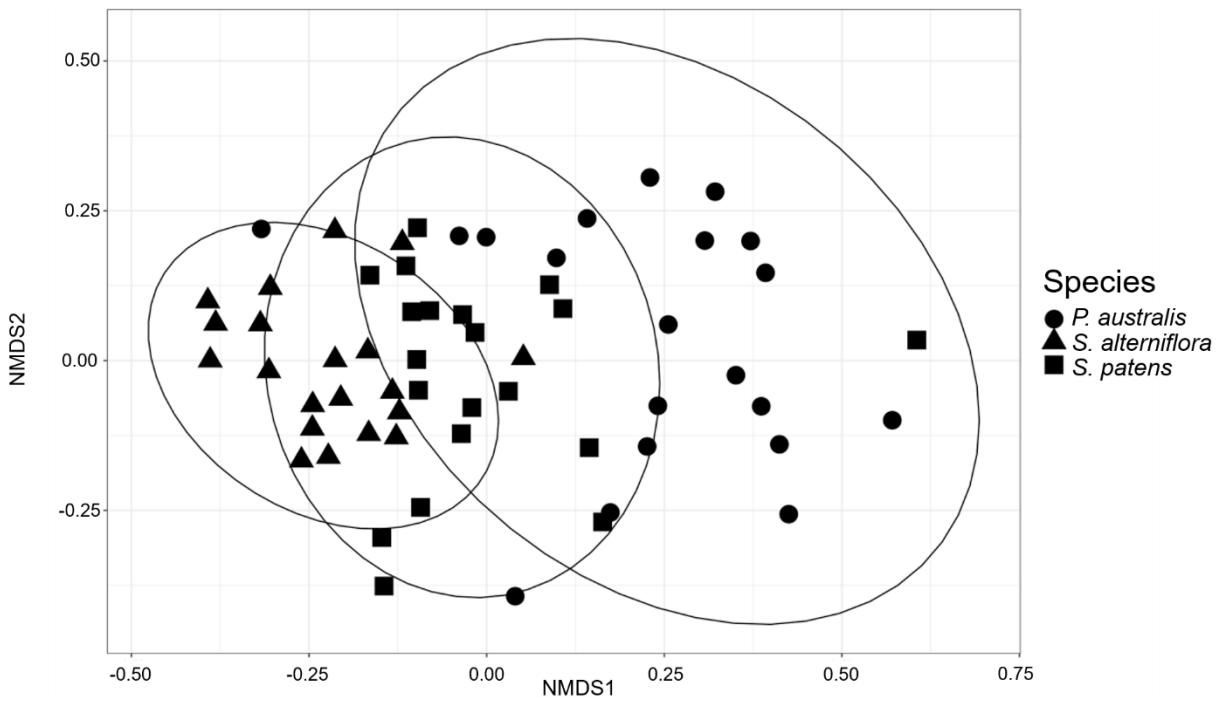

Figure 4. Non-metric multidimensional scaling plot of ordinal distances between 60 sediment samples for microbial communities (20 sites x three vegetation zones) with a stress level of 0.19. Ellipses represent a 95% confidence interval around each vegetation zone based on a multivariate t-distribution. Ellipses demonstrate that the structure of microbial communities may be influenced by the conditions of each of the dominant vegetation zones.

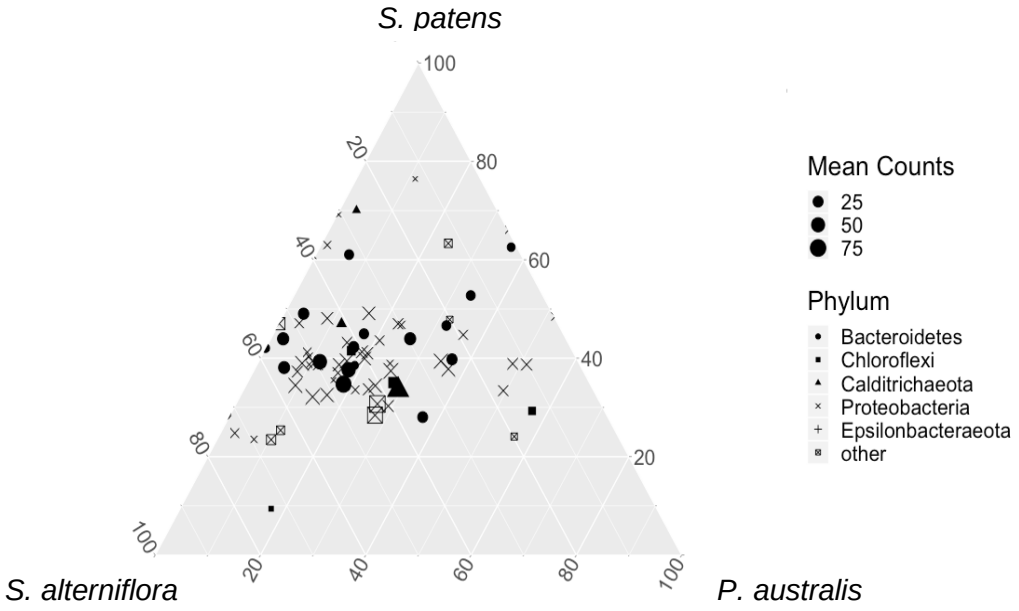

Figure 5. Ternary plot representing relative abundance of operational taxonomic unites (OTUs) among the three vegetation zones. The 500 most abundant OTUs are displayed based on the phylum to which they were classified (the five most common phyla are indicated, with remainder assigned to "other"). The size of the point indicates the mean count of each OUT. Only OTUs with at a sequence count of at least 10 were retained to examine OTUs that could potentially be shared between all three zones. Bacteroidetes and Proteobacteria phyla were often the differentiating phyla among vegetation zones.

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Salt marsh plant-mediated carbon turnover overrides effects of sea-level rise

Abstract

To determine how sea-level rise (SLR) may impact carbon cycling rates among dominant salt marsh vegetation zones, we manipulated marsh elevation and vegetation composition using a marsh organ experiment. We quantified CO_2 fluxes (net ecosystem exchange (NEE); ecosystem respiration (ER)) and soil carbon mineralization rates in response to three SLR-scenarios (present day, ~10-year SLR (+7.5cm), ~20-year SLR (+15cm)) and five vegetation treatments (*Spartina alterniflora, Spartina patens, Phragmites australis*, two unvegetated controls). SLR treatments increased NEE with reduced carbon uptake at both 10-year and 20-year levels compared to present day, but interestingly other carbon flux metrics and soil parameters (electrical conductivity, soil moisture, SO_4^-, Cl^-, NH_4^+) were not responsive to our SLR treatments. In contrast, our vegetation treatments affected all carbon flux measurements; *S. alterniflora* and *S. patens* had greater CO_2 uptake and ER compared to *P. australis*. Soil carbon mineralization assays indicated that soils associated with *Spartina* spp. emitted more CO_2 than *P. australis* or unvegetated controls. As marshes flood more frequently with projected SLR, marsh vegetation composition is predicted to shift towards more flood-tolerant *Spartina* spp., which may lead to increased carbon turnover rates. While hydrologic conditions and tidal flow may influence the location of marsh vegetation, our findings suggest that plants, more so than incremental flooding, play a critical role in driving carbon cycling within a salt marsh.

Introduction

Salt marshes are located at the unique boundary between terrestrial and marine ecosystem (McLeod et al. 2011) and are valued for nutrient removal, fish nurseries, and storm mitigation (Bromberg & Bertness 2005; Barbier et al. 2011). Along with mangroves and seagrass beds, coastal wetlands have high carbon sequestration rates and are recognized as "blue carbon ecosystems" (McLeod et al 2011) whose conservation and restoration are increasingly targeted to mitigate climate change (Pendleton et al. 2012); however they are threatened due to their limited geographic extent and urbanization of coastlines. In temperate regions, salt marshes have persisted along tidally flooded continental margins for thousands of years (Redfield 1965) and are dominated by productive graminoids capable of high rates of CO_2 uptake, which coupled with anoxic soils promotes the accumulation of organic matter (Kirwan and Megonigal 2013). In this era of rapid sea-level rise (SLR) however, there is great concern about the future of salt marshes. Historically marshes were able to "migrate" landward in response to SLR, but coastal development limits landward migration and promotes marsh drowning (Enwright et al. 2016; Field et al. 2016). Salt marshes of the northeastern United States may be particularly vulnerable to SLR-induced changes, as between 1980-2009 SLR rates were 3-4 times greater than the global mean (Sallenger et al. 2012; Horton et al. 2014), and it is predicted that up to 9.5% of developed upland will become regularly flooded in the next 30 years (Clough et al. 2015). For example, 17% of Rhode Island marshes have been lost to SLR in the past four decades (Watson *et al.* 2017).

Rapid increases in flooding alters marsh composition, as vegetation is highly responsive to hydrologic conditions (Bertness 1991; Crain et al 2009; Smith & Warren 2012). As flooding increases, low marsh vegetation replaces high marsh zones; areas once dominated by *Spartina*

patens and *Juncus gerardii* have transitioned to stunted *Spartina alterniflora* (Warren & Niering 1993; Field et al. 2016). Of the 5900 hectares of marsh remaining in Connecticut, nearly two-thirds are dominated by *Spartin*a spp., primarily *S. alterniflora* and to a lesser extent *S. patens* (Rosza 2012). *Spartina alterniflora* is better suited to tolerate frequent flooding than high marsh vegetation because it allocates greater resources belowground in the form of roots and rhizomes (Bromberg & Bertness 2005), and can effectively mitigate reduced sediment by exuding oxygen into its rhizosphere (Bertness 1991). Brackish species including the invasive *Phragmites australis* thrive along the upland marsh boundary where flooding is infrequent.

While differences in carbon cycling among salt marsh zones have been documented (Martin & Moseman-Valtierra 2015; Barry et al. *in prep*), it is unclear whether they are propelled by biotic inputs from vegetation or by abiotic tidal flooding. Wetland plants can alter the sediment environment through a variety of processes that could alter microbial competition for organic carbon. Aerenchymous tissues and pressurized ventilation can translocate oxygen from the atmosphere to the rhizosphere, which can stimulate microbial decomposition of organic matter (Freeman *et al.* 2001; Sutton-Grier & Megonigal 2011). Low-molecular weight, labile carbon compounds exuded by plants into the rhizosphere (Farrar et al. 2003) are quickly metabolized by microbial communities. Soil organic matter decomposition is also driven by physiochemical conditions such as flooding and salinity. Increased sulfate concentrations in soils due to increased tidal flooding may enhance sulfate-reduction and carbon mineralization rates (Chambers et al. 2011; Simon et al. 2017). However, the effects of salinity on wetland soil respiration is not consistent; while salinity and soil respiration were positively correlated in a long-term experimental manipulation in freshwater tidal wetlands (Weston *et al.* 2011) and in a field survey of 20 coastal salt marshes (Barry *et al. in prep*), others have found decreased CO_2

production with salinity in brackish tidal wetlands (Doroski et al. 2019). This discrepancy may partly be due to the lack of incorporating vegetation in experimental design and the indirect effects plants can have on mediating soil respiration.

To disentangle two key drivers of salt marsh carbon cycling, plant mediated inputs (biotic) and flooding frequency (abiotic), we experimentally manipulated plant composition and elevation using a marsh organ experiment (*sensu* Morris 2007) in a tidal creek in southern New England. While previous studies have projected longer-term SLR (i.e., decades to centuries; Langley et al. 2009; Simon et al. 2017), we chose to investigate the effect of projected flooding associated with near-future SLR scenarios (i.e., ~10 and 20 years in the future) using SLR projections from the Connecticut Sea Level Affecting Marshes Model (SLAMM 6) (Clough *et al.* 2015). We hypothesized that (i) elevation manipulations that increase flooding and salinity would drive soil respiration rates rather than vegetation because previous studies (Barry et al. *in prep*; Weston et al. 2011; Chambers et al. 2011; Marton et al. 2012) suggest positive correlations between soil salinity and carbon dioxide (CO_2) production; (ii) low-elevation, more salt-tolerant species (*S. alterniflora*) would be more resilient to increased flooding whereas the less tolerant, higher-elevation species (*P. australis*) would be more susceptible to senescence and have reduced CO_2 uptake (i.e., net ecosystem exchange) and higher respiration rates.

Methods

Study site- We selected the primary tidal creek in Impoundment 3 of Barn Island Wildlife Management Area in Stonington, CT, USA (41°20'27.4"N 71°51'56.4"W) to install our experiment, as it was wide (~4m) enough to accommodate our experimental design, and had a consolidated, level bottom. The tidal creek was restricted by the construction of a road in 1947, but in 1976 aluminum squashed culverts were installed to reconnect the creek to the estuary (Ron Rosza, *personal communication*). Because of the previous restriction as well as the presence of

ground water seepage upstream (Roger Wolfe, *personal communication*), all three target vegetation species (*S. alterniflora*, *S. patens*, *P. australis*) were present in the impoundment. Surface water salinity during site selection (March 2018) suggested the wetland was mesohaline (3-9ppt), but salinity increased to polyhaline (18-25ppt) conditions during the growing season.

Experimental approach-"Marsh organ" experiments (*sensu* Morris 2007; Kirwan & Guntenspergen 2012) simulate various levels of marsh elevation at a single location to isolate the effect of hydroperiod, which is often confounded by salinity and soil composition along environmental transects (Morris 2007). To assess how flooding associated with different SLR scenarios affected plant biomass allocation, soil properties, as well as autotrophic and heterotrophic carbon cycling responses, we employed a full-factorial marsh organ experiment, testing five vegetation (*S. alterniflora, S. patens, P. australis*, unvegetated low marsh, unvegetated high marsh) and three SLR scenario (Present Day, 10-Year SLR, 20-Year SLR) treatments that were replicated via five platforms. Each wooden-framed platform contained 15 (5 vegetation x 3 SLR treatments) mesocosms, which were constructed from 6-inch (15cm) diameter PVC pipes cut to varying heights to simulate different flooding scenarios.

To determine appropriate pipe heights that would simulate ecologically relevant SLR scenarios for the three species of interest, we used survey leveling techniques to quantify the relative differences in elevation among the three target vegetation zones. We surveyed 15 random points (five per zone) in Impoundment 3 using an auto level and stadia rod, and determined that *S. alterniflora* zones were 10-cm lower in elevation on average than zones dominated by *S. patens* or *P. australis*. Therefore, we cut PVC pipes for "low marsh" treatments (*S. alterniflora*, low marsh unvegetated) 10-cm lower than "high marsh" (*S. patens, P. australis*, high marsh unvegetated). We also manipulated the lengths of pipes to test three SLR-scenarios

(shorter pipes: more frequently flooded). The present-day (2018) treatment was the tallest, and we reduced the pipe length by 7.5cm and 15cm to simulate 10-Year SLR and 20-Year SLR flooding scenarios, respectively, based on the Global Climate Model Maximum SLR estimates for the region by Clough et al. (2015).

Installation- The five platforms (each ~1.5 x 1-m) were installed within a 10-m stretch of the tidal creek, with ~1m of separation between them (Fig. 1). We oriented platforms in randomly determined directions (0-360°) to reduce potential bias from shading, and randomly located the 15 treatment combinations within each platform. Pipes were pounded into tidal creek sediment to the appropriate elevation using a rubber mallet, level, and surveying equipment at the end of April 2018, and treatment pots were inserted into the tops of pipes on May 21, 2018. For vegetated treatments, we excavated ~10x10x10cm sections of sediment (including belowground biomass) from areas dominated (>50% cover) by the three target species in Impoundment 3. For each species, we attempted to visually equalize aboveground biomass and recorded maximum stem height and density of all transplants; *S. alterniflora* and *S. patens* shoots were ~30-cm tall with 20-30 stems, whereas *P. australis* stems were 50 to100-cm tall and had three live stems per transplant. We filled the bottom 20 cm of each pot (15-cm diameter x 30-cm tall, with two, 2-cm wide holes at the bottom) with a 2:2:1 sediment mixture consisting of sand, sphagnum peat moss, and 5-mm sieved tidal creek sediment, respectively; we placed transplants on top and filled in gaps with our sediment mixture. Our preliminary pilot studies of pure tidal creek sediment suggested poor drainage due to high clay content, so we amended with sand and peat moss to achieve drainage between tidal cycles. Potted plants and unvegetated replicates completely filled with our 2:2:1 sediment mixture were placed into vertical PVC pipes, with pot lips flush with the top of the PVC pipes. To prevent slipping and maintain pots at treatment elevations, we tied two

pieces of rope through holes drilled into each PVC pipe to support pot bases. Two additional 1.5-cm diameter holes were drilled 10-cm beneath pot bases to ensure drainage; thus, sediments were subjected to flooding from below as well as when creek levels overtopped the lip of pipes. To minimize algal deposition on mesocosms, we installed plastic mesh drift fences on either side of the five platforms and removed trapped algae weekly.

Field Sampling- To monitor hourly water depth and salinity (ppt) over the course of a tidal cycle, we installed an In-Situ® Aqua TROLL® 200 (In-Situ, Fort Collins, CO) from July 20 to September 1, and used barometric pressure readings from NOAA Station NLNC3 - 8461490 - New London, CT. In mid- and late-August, we conducted gas flux sampling campaigns to estimate net ecosystem exchange (NEE, using transparent chambers) and ecosystem respiration (ER, using opaque chambers) using a Picarro G2201-*i* cavity ring-down spectrometer (Santa Clara, CA). We strategically sampled gas fluxes on sunny days late in the growing season when low tide occurred at 1200; all measurements were collected between 0900 and 1500. During sampling campaigns, we measured photosynthetically active radiation (PAR) using a Hobo Micro Station H21-USB (Bourne, MA) placed adjacent to platforms On August 6-8, 2018 we sampled CO_2 flux from each mesocosm during 10-minute transparent chamber incubations. Methane was also measured, but due to the high salinity in the creek, CH_4 fluxes were negligible. On August 20-23, 2018 we estimated ER during 10-minute dark chamber incubations from a subset of our mesocosms; we sampled the present day (n = 25) and 20-year SLR (n = 25) treatments to examine how our extreme SLR treatments affected ER.

Biomass- On May 21 and August 13, 2018, we quantified stem densities, average stem height, and maximum stem height for each vegetated mesocosm. After more than four months, we ended the experiment on September 1, 2018; pots were extracted from PVC pipes, placed into plastic

bags, and transported to the University of Connecticut Storrs for laboratory analyses. Aboveground stems were clipped at the soil surface, dried (65℃ for ≥48 hours), and weighed. The top 10cm of each pot was cut with a reciprocating saw and then halved vertically; to estimate belowground biomass, one half was wet-sieved using a garden hose and nozzle over a 2-mm screen to separate roots and rhizomes from the interstitial sediment, and the second half was used for sediment and soil wet chemistry. Roots and rhizomes were dried (65℃ for ≥48 hours) and weighed.

Sediment & Soil Wet Chemistry- To quantify gravimetric soil moisture, a subsample (~5g) was dried at 105°C for 72 hours. We then estimated loss on ignition (LOI) on the subsample by combusting organic matter at 550°C for four hours. Another subset of dried sediment was pulverized using a ball mill and analyzed for %C and %N content using a Costech ECS 4010. Soil slurries (1:5 ratio of soil to deionized water) were used to determine soil electrical conductivity ($EC_{1:5}$) and $pH_{1:5}$ which were measured with an Orion Conductivity Cell and an Orion Star A215 pH Conductivity Meter Orion with Ross Ultra pH/ATC Triode at room temperature (25°C). We analyzed water extracts for chloride (Cl^-) and sulfate (SO_4^{2-}) on a Dionex Ion Chromatography System (ICS)-1100 (Thermo Fisher Scientific, Waltham, MA). Samples of 2.5g soil with 25 ml DI water were shaken for 30 minutes at 200 rpms and then centrifuged at 2500 rpms for five minutes. The supernatant was filtered through Whatman GF/F filters prior to being run. We extracted soil-water ammonium (NH_4^+-N) with 2M KCl (soil:KCl = 1:10), filtered extracts using 110mm Whatman paper (adapted from Keeney and Nelson 1982), and analyzed them on a SmartChem®200 discrete analyzer (Westco Scientific Instruments, Brookfield, CT).

Carbon Mineralization- Carbon mineralization rates were measured as headspace-accumulated CO_2 during a 24-hour period utilizing a Picarro G2201-*i* gas analyzer and two, 16-port

distribution manifolds (Johnson 2018). We created soil slurries by adding ~50g of sieved soil with 100mL of deionized water to 196 mL glass canning jars, allowed them to come to room temperature, and connected them to the gas analyzer; headspace gas concentrations were measured approximately every two hours over a 24-hour period. We calculated gas flux rate as the linear change in concentration over time. We also corrected for chamber temperature, atmospheric pressure, and chamber volume, based on the ideal gas law.

Statistical Analyses- We tested all response variables for normality using Shapiro-Wilk tests, assessed heteroscedasticity by plotting fitted values against residuals, and achieved normality by log transformations when necessary. We conducted t-tests between unvegetated and vegetated treatments; however, because our unvegetated treatment sediments were 100% comprised of our custom 2:2:1 soil mixture and vegetated treatment sediments were ~50:50 soil mixture to native sediment, we concluded that the unvegetated treatments were not true controls and omitted them from further analyses. We conducted two-way analysis of variance (ANOVA) to test for differences among species, SLR scenario, and their interaction. We did not observe platform effects for any of our parameters. We ran a repeated measures ANOVA for stem measurements taken in May compared to August (*lsmeans* package). When categorical factors were significant ($\alpha = 0.05$), we ran post-hoc Tukey HSD tests. All statistical analyses were run in R version 3.5.1 (R Core Team 2017).

Results

Tidal Regime- From July 20 to September 1 2018, the mean daily tidal range was 0.64m ($\pm$ 0.07m), creek salinity ranged from 16-26ppt, and water temperatures ranged from 21-29°C. Depending on vegetation and SLR treatments, mesocosms were differentially inundated (i.e., water level above the lip of the pipe). As expected, present day SLR treatments were the least

flooded pots whereas 20-Year SLR treatments experienced the greatest amount of flooding. *Spartina alterniflora* experienced greater inundation (38, 44, and 51% of the time) for present day, 10-Year SLR, and 20-Year SLR, respectively, than either *S. patens* or *P. australis* (27, 36, and 43% of the time). Pot bases were submerged more frequently; for present day, SLR 10-Year, and SLR 20-Year treatments, respectively, *S. alterniflora* bases were submerged for 65, 75, and 88% of the time, and *S. patens* and *P. australis* treatments were submerged 55, 62, and 72% of the time.

Soil Chemistry- Soil chemistry parameters ($EC_{1:5}$, Cl^-, SO_4^{2+}, % soil moisture, pH, % organic matter) were all significantly greater in vegetated than unvegetated treatments, except for organic matter which was greater in unvegetated mesocosms (Table 1). Surprisingly, neither SLR nor species treatments effected any measured soil chemistry parameters (Table 2). Ammonium concentrations were omitted from analyses as only four of 89 samples were above detection limit (1.58 ppm).

Plant Response- From May to August 2018, we observed a significant interaction between SLR and species treatments for stem density ($F_{4, 76} = 9.90$, $p < 0.001$) but we only observed an effect of species on stem height ($F_{2, 84} = 70.35$, $p < 0.001$) and no effect of SLR on stem height. *Spartina alterniflora* stem density dropped from an average of 33 to 22 stems per mesocosm over the experiment, but average height increased from 27cm to 39cm. *Spartina patens* stem density increased from of 41 to 118 stems, but stem height was consistent (~24cm). *Phragmites australis* stem density increased from 3 to 6, but average height decreased from 67cm to 33cm; we observed *P. australis* shoot senescence and new shoot development throughout the growing season. We did not observe differences in above- or belowground biomass due to SLR treatment, but aboveground biomass differed among species; *S. alterniflora* had greater aboveground

biomass (916.5 ± 84.5 g m^{-2}) than *S. patens* (639.8 ± 59.7 g m^{-2}) and *P. australis* (458.8 ± 54.2 g m^{-2}; Table 3).

Net Ecosystem Exchange (NEE)- Average PAR during our three-day *in-situ* field campaign was 1350 ± 14 µmolE m^{-2} min^{-1}. We did not include PAR as a covariate in our analyses as it was not correlated with NEE (R^2 = 0.05). *In-situ* soil temperature and soil conductivity also poorly described net ecosystem exchanges rates (R^2 = 0.02 and R^2 = 0.01 respectively). We found a significant positive correlation between aboveground biomass and net ecosystem exchange across species (R^2 = 0.29, p <0.001). We found the correlations to differ when we separated NEE rates based on vegetation zones. The greatest R^2 value corresponded to *S. patens* (R^2 = 0.41), then *S. alterniflora* (R^2 = 0.11) and no correlation was found for *P. australis* (R^2 = 0.00)

We observed significant effects of SLR ($F_{2, 40}$ = 17.67, p < 0.001) and species treatments ($F_{2, 40}$ = 11.82, p < 0.001), but no significant interaction. For each species, greatest CO_2 uptake occurred at present day elevations, whereas greatest CO_2 emissions occurred in the 20-Year SLR treatments (Fig. 2). *Spartina alterniflora* had the greatest carbon uptake in 10-Year SLR treatment with -5.13 ± 1.28 µmol CO_2 m^{-2} s^{-1}, whereas the greatest carbon emissions was *S. patens* at the 20-Year SLR treatment with 5.21 ± 1.17 µmol CO_2 m^{-2} s^{-1}. *Phragmites australis* was not a carbon sink under any SLR treatment.

Ecosystem Respiration (ER)- We did not observe SLR treatment effects on ER, however respiration rates differed among species ($F_{2, 26}$ = 7.6, p <0.01). Respiration rates from *S. alterniflora* (10.48 ± 1.10 µmol CO_2 m^{-2} s^{-1}) were greater than *P. australis* (5.40 ± 0.67 µmol CO_2 m^{-2} s^{-1}), while *S. patens* (8.54 ± 0.94 µmol CO_2 gC^{-1} s^{-1}) respiration rates were similar to both species (Fig. 3). We were unable to combine NEE and ER to quantify gross primary production, as soil temperatures differed considerably between gas sampling campaigns (NEE:

33°C vs ER: 27°C). We found a positive correlation between aboveground biomass and ER across species ($R^2 = 0.13$, $p = 0.05$). We found the correlations to differ when we separated ER rates based on vegetation zones. The greatest R^2 value corresponded to *P. australis* ($R^2 = 0.35$), then *S. patens* ($R^2 = 0.25$), then *S. alterniflora* ($R^2 = 0.23$)

Carbon Mineralization- We did not observe differential carbon mineralization rates among SLR treatments, but observed differences among species ($F_{2, 40} = 4.85$, $p < 0.05$). Carbon mineralization from *S. patens* sediments (19.5 ± 2.3 µmol CO_2 gC^{-1} hr^{-1}) were greater than those from *P. australis* (12.5 ± 1.2 µmol CO_2 gC^{-1} hr^{-1}), while *S. alterniflora* (16.2 ± 1.3 µmol CO_2 gC^{-1} hr^{-1}) rates were intermediate (Fig. 4). We observed a weak but positive correlation between belowground biomass and carbon mineralization ($R^2 = 0.07$, $p = 0.05$). We found the correlations to differ when we separated carbon mineralization rates based on vegetation zones. *Spartina patens* had the greatest correlation ($R^2 = 0.29$) and neither *S. alterniflora* nor *P. australis* had a strong correlation, ($R^2 = 0.01$ for both species). There was also a positive correlation between chloride concentration and carbon mineralization ($R^2 = 0.10$, $p < 0.05$). We found *S. alterniflora* to have the highest correlation ($R^2 = 0.35$) when we separated carbon mineralization rates by species whereas *S. patens* ($R^2 = 0.16$) and *P. australis* ($R^2 = 0.11$) had lower correlations.

Discussion

Our study tested the influence of plant species and near-future SLR effects on carbon cycling in coastal wetlands. Using a marsh organ experiment in a tidal creek in southeastern Connecticut, we manipulated three coastal marsh grass species and their elevations based on SLR projections from SLAMM (Clough et al. 2015). While we expected carbon-based processes to be more responsive to SLR than vegetation treatments because flooding dictates vegetation zonation in salt marshes (Morris et al. 2002), we only observed differences in NEE among SLR

treatments, whereas ER and carbon mineralization rates were similar among SLR treatments. We found support for our prediction that flood-tolerant *S. alterniflora* would be most resilient to increased flooding, as it had the largest uptake of carbon (NEE) among vegetation treatments at present day and 10-Year treatment levels of SLR. Surprisingly, at the conclusion of our four-month experiment, we did not observe differences in soil chemistry ($EC_{1:5}$, $pH_{1:5}$, SO_4^{2+}, Cl^-, soil moisture, LOI, %C, %N) among SLR nor vegetation treatments, yet we observed differences in carbon fluxes among vegetation treatments. Our findings suggest differences in carbon cycling were more plant-mediated than by hydrological modifications.

Plant Mediated Responses- Our unvegetated treatments, although different in soil composition than vegetated treatments, highlight the priming effect that plants have on microbial communities (Wolf et al. 2007). NEE and ER were both an order of magnitude higher in vegetated than unvegetated treatments, highlighting the additional autotrophic uptake and respiration contributed by vegetation, and carbon mineralization (standardized by soil carbon content) was twice as high in vegetated treatments. Similarly, Mueller et al. (2016) found that salt marsh vegetation can stimulate soil organic matter decomposition up to 260%. Plants contribute to decomposition by supplying terminal electron acceptors (Wolf et al. 2007) and regenerating reduced forms of nitrogen, sulfur, and iron (Sutton-Grier and Megonigal 2011), as well as exude low molecular weight carbon from roots (Blagodatskaya and Kuzykov 2008). We sampled fluxes within three hours of low-tide on consecutive days in the growing season with similar conditions (sunny and air temperature ~25°C); however there may have been other environmental conditions we did not consider. For example, Artigas et al. (2015) found that only 5% of carbon uptake in a New Jersey salt marsh was explained by changes in water level whereas 66% of the variation was explained by time of day and air temperature.

Increased flooding may not affect all aspects of salt marsh carbon cycling. Our SLR treatments reduced NEE but not ER or carbon mineralization. Vegetated treatments declined by 50% in NEE from present day to 10-Year SLR and became a source of carbon at 20-Year SLR at nearly the same rate at which they were a sink at present day treatments. In Virginia, *S. alterniflora* photosynthetic rates declined as much as 66% under flooded conditions due to limited light availability and reduced efficiency resulting in a 46% decrease in NEE (Kathilankal et al. 2008). Previous studies investigating NEE associated with marsh vegetation using eddy-covariance (Artigas et al. 2015) or static chambers (Cornell et al. 2007) found similar to slightly higher rates of NEE than our estimates, which perhaps were lower because of high salinity and inundation frequency in the tidal creek (Nuttle and Hemond 1988). Our NEE estimates were restricted to a three-day campaign during the peak of the growing season, but evidence from Artigas et al. (2015) suggests that phenological differences among species may be important to consider, as they found greater NEE uptake in *S. patens* than low marsh species because it had a longer photosynthetic period during the growing season. In contrast, Cornell et al. (2007) observed similar field NEE rates in *S. alterniflora* to our experiment. While Martin & Moseman-Valtierra (2015) were not investigating SLR effects, they found native *Spartina* spp. to have greater carbon uptake than *P. australis.* It is important to note that we initially chose our experimental because it was a mesohaline marsh, however as the summer months progressed, the salinity increased above the threshold that *P. australis* can typically tolerate (Chambers et al. 1999) and we observed senescence and low vigor.

Greater ER and carbon mineralization rates in *Spartina* spp. than *P. australis* may be due to species effects on rhizospheric redox conditions. Roots contribute additional terminal electron acceptors and oxygenate soils (Wolf et al. 2007; Sutton-Grier & Megonigal 2011), therefore

belowground biomass has been linked to higher soil respiration (Barry et al. *in prep*) and carbon flux (Moseman-Valtierra et al. 2016). In a previous study, we found higher belowground biomass in *Spartina* spp. than *P. australis* (Barry et al. *in prep*), with elevated root respiration likely contributing to higher rates of carbon mineralization. Further, *S. alterniflora* respires new photosynthate quickly, as Spivak & Reeve (2015) found *S. alterniflora* to respire 30-55% of fixed carbon within 24 hours. We implemented 24-h slurried incubations, which quantify microbial utilization of labile readily available carbon, whereas Craft et al. (2003) and Cornell et al. (2007) ran long-term (76 days) slurried incubations under anaerobic conditions to quantify both labile and more recalcitrant fractions. We considered short-term 24-h incubations to be more representative of semi-diurnal tidal conditions as hydrologic conditions in a salt marsh can be altered significantly, even in a matter of hours. Although we did not directly quantify rhizospheric microbial communities, plants can exert strong influences on their microbial assemblage (Rietl et al 2016; Barreto et al. 2018). Previous SLR experiments found sulfate-reducer abundance increases as soils are subjected to more frequent or intensive salt-water flooding and increase CO_2 respiration (Hines et al. 1999; Marton et al. 2012). This corroborates our findings that the more frequently flooded soils of *S. alterniflora* had higher rates of carbon mineralization and ER, which may be in part due to increased sulfate-reducers and the species ability to oxygenate soils.

SLR & Management- Increased flooding frequency is shifting the areal extent of vegetation (Carey et al. 2015; Field et al. 2016) and subsequently affecting carbon cycling (McLeod et al. 2011). In coastal New England, increased flooding associated with SLR may facilitate further dominance of *S. alterniflora* and squeeze out less salt-tolerant species such as *S. patens* (Warren & Niering 1993; Doody 2004; Field et al. 2016). While we did not detect differences in ER and

carbon mineralization between *S. alterniflora* and *S. patens*, each species provides unique and valuable ecosystem services. For example, high marsh species *Juncus gerardii* and *S. patens* provide nesting habitat for critically endangered salt marsh sparrow (Bayard & Elphick 2011). In our 20-Year SLR treatment, *S. patens* respired more CO_2 than *S. alterniflora*, suggesting in the next 20 years as marsh vegetation shifts, this ecosystem may lose both carbon storage and biodiversity services. Previous studies suggest *P. australis*-dominated soils have greater carbon uptake and respire less carbon than native vegetation, most likely due to abundant aboveground biomass and recalcitrant soil carbon (Windham 2001; Martin & Moseman-Valtierra 2015). In contrast, we observed higher respiration rates in *P. australis* than *Spartina* spp. treatments, perhaps due to the inability of *P. australis* to tolerate salinity levels >18ppt (Chambers et al. 1999) or because transplants were stressed after being severed from their clonal network (Amsberry et al. 2000). While our experiment may not have been the ideal environment for *P. australis*, our NEE results nonetheless demonstrate the decreased capabilities of plants to effectively sequester carbon with SLR. Prolonged inundation generates numerous stressors upon vegetation including oxidative stress brought on by reduced soil conditions, interstitial salinity stress, and accumulation of phytotoxic byproducts (Langley et al. 2013). Reponses to flooding can vary depending on species and flooding severity, but previous studies indicate a negative feedback interaction between increased flooding and decreased belowground biomass (Kirwan and Guntenspergen 2012; Watson et al. 2016). As SLR and coastal development reshape salt marsh communities, understanding how best to manage vegetation to maintain the efficiency of blue carbon ecosystems is necessary. Using vegetation as biological and biogeochemical indicators may prioritize the preservation of more flood-sensitive vegetation in the face of SLR.

We suspect that that our elevation treatments were not substantially different enough to trigger differences. We manipulated elevations to simulate 10 and 20 year SLR scenarios, however all the pots were saturated at similar rates. The tops of all mesocosms were flooded at different rates during high tide (27-51% of the time), flooding from the bottom of pots resulted in saturation at least 55% of the time for all treatments. Our SLR treatments did not alter the suite of soil chemistry parameters we quantified ($EC_{1:5}$, $pH_{1:5}$, SO_4^{2+}, Cl^-, soil moisture, LOI, %C, %N) nor two respiration indices (ER and carbon mineralization). We assumed that manipulating the elevation of vegetation grown in a tidal creek would be reflective of future SLR scenarios in southeastern Connecticut; however it is uncertain how else hydrodynamics may be effected with rising sea-level. Soil depth in our mesocosms was limited to 30cm, whereas extant vegetation in the marsh grow in deep (>1m) organic soils. Mozdzer et al. (2016), found *P. australis* to accelerate the decomposition of deep, recalcitrant organic matter at greater depths than native vegetation. The majority of belowground biomass of native vegetation occurred in the top 30cm whereas *P. australis* has a below- to aboveground biomass ratio that exceed 3:1. Surface area was also limited in our 15-cm wide pots reducing horizontal flow of sea water, whereas in a natural marsh tidal waters are able to disperse over a large lateral extent. Despite our mesocosms having several drainage holes, soil drainage was likely dissimilar to natural marshes (Kirwan & Guntenspergen 2012).

Future Directions- This experiment manipulated both vegetation and elevation to investigate the relative roles of dominant plant species and projected SLR-induced flooding on salt marsh carbon cycling. Salt marshes play a critical role in mitigating climate change via carbon sequestration (McLeod et al. 2011) and vegetation can be a useful indicator of carbon and nitrogen cycling (Barry et al. *unpublished*; Ooi et al. *unpublished*). Our results suggest that

carbon cycling is plant-mediated more than near-future changes in flooding associated with SLR. Plants contribute to carbon cycling primarily through aboveground carbon uptake and priming of soils (Mueller et al. 2016). Future efforts should assess rhizospheric microbial communities and root exudates to better evaluate how root exudates and oxygenation of soils by influences microbial activity. Previously, we found belowground biomass to be a primary driver of soil respiration (Barry et al. *in prep*) and Moseman-Valtierra et al. (2016) suggested using belowground biomass as a proxy for coastal managers to estimate CO_2 fluxes. Accretion in salt marshes is primarily driven by sediment capture and organic matter accumulation (Warren & Niering 1993). However, as vegetation shifts in response to increased SLR, it is uncertain how soil previously inhabited with less salt-tolerant plants, or soil legacies, will impact biogeochemical cycles. Ultimately, researchers and managers should continue to monitor shifts in vegetation composition across southern New England and conserve these valuable ecosystems.

Table 1. Soil chemistry parameters and carbon cycling rates averaged across unvegetated (high and low marsh) and vegetated (*S. alterniflora*, *S. patens*, *P. australis*) treatments. Log-transformed data were used for t-tests, but untransformed averages (±1 SE) are presented here. Differences between unvegetated and vegetated treatments are denoted. In general the presence of vegetation tends to increase values associated with soil chemistry and the magnitude of carbon cycling rates.

	Unvegetated	**Vegetated**
$EC_{1:5}$ (mS cm^{-1}) *	4.52 (0.12)	5.04 (1.90)
Chloride (mg kg Dry $Soil^{-1}$) ***	41.57 (3.25)	67.28 (4.84)
Sulfate (mg kg Dry $Soil^{-1}$) ***	4.96 (0.45)	7.86 (0.64)
$pH_{1:5}$***	5.54 (0.07)	6.00 (0.05)
% Soil Moisture ***	73.27 (6.23)	172.77 (12.16)
% Organic Matter	17.45 (1.41)	16.48 (0.91)
% C ***	6.26 (0.17)	10.84 (0.47)
% N ***	0.13 (0.003)	0.51 (0.04)
NEE (µmol CO_2 m^{-2} s^{-1})**	1.95 (0.17)	-0.17 (0.62)
ER (µmol CO_2 m^{-2} s^{-1})***	1.30 (0.23)	8.14 (0.64)
Carbon Mineralization (µmol CO_2 gC^{-1} hr^{-1})***	7.53 (0.65)	16.09 (1.03)

NS: $p > 0.05$
*: $p < 0.05$
**: $p < 0.01$
***: $p < 0.001$

Table 2. Soil chemistry parameters averaged across vegetated (*S. alterniflora*, *S. patens*, *P. australis*) and SLR treatments. Averages (± 1 SE) are presented here. No significant differences were observed for soil chemistry parameters between species or SLR treatments.

	Species Treatment				**SLR Treatment**		
	S. alterniflora	***S. patens***	***P. australis***		**Present Day**	**10-Year SLR**	**20-Year SLR**
$EC_{1:5}$ (mS cm^{-1})	4.79 (0.30)	5.31 (0.36)	5.00 (0.32)		5.19 (0.32)	5.07 (0.30)	4.85 (0.36)
Chloride (mg kg Dry $Soil^{-1}$)	71.11 (9.26)	67.32 (6.14)	63.41 (9.74)		64.68 (6.23)	76.38 (8.94)	60.79 (9.61)
Sulfate (mg kg Dry $Soil^{-1}$)	8.15 (0.85)	7.85 (1.33)	7.58 (1.17)		7.62 (0.95)	9.02 (1.11)	6.93 (1.25)
% Soil Moisture	174.00 (21.56)	180.10 (19.52)	164.21 (23.20)		155.02 (17.47)	201.03 (26.18)	162.27 (17.79)
$pH_{1:5}$	6.17 (0.09)	5.96 (0.09)	5.87 (0.08)		5.9 (0.11)	6.07 (0.08)	6.02 (0.08)
% Organic Matter	16.66 (1.51)	18.02 (1.90)	14.76 (1.21)		16.10 (1.47)	17.32 (1.91)	16.01 (1.36)
% C	11.18 (0.83)	10.52 (0.61)	10.82 (3.83)		11.04 (0.95)	11.44 (0.87)	10.04 (0.58)
% N	0.57 (0.07)	0.49 (0.05)	0.48 (0.08)		0.05 (0.08)	0.56 (0.07)	0.45 (0.05)

Table 3. ANOVA results of parameters representing biomass and carbon flux rates. There was no statistical effect of SLR treatments for any of the parameters listed below except for NEE. There was no significant interaction (Species*SLR) for any of the parameters listed below. (NS: $p > 0.10$, *: $p < 0.05$, **: $p < 0.001$, ***: $p < 0.001$).

Response	Treatment	*df*	F-value	p-value
Aboveground Biomass ($g\ m^{-2}$)	Species	2	11.83	***
	SLR	2	1.26	NS
log Belowground Biomass ($g\ m^{-2}$)	Species	2	0.73	NS
	SLR	2	0.53	NS
NEE	Species	2	12.73	***
	SLR	2	19.04	***
ER	Species	2	7.6	**
	SLR	1	0.71	NS
Carbon Mineralization	Species	2	4.85	*
	SLR	2	2.31	NS

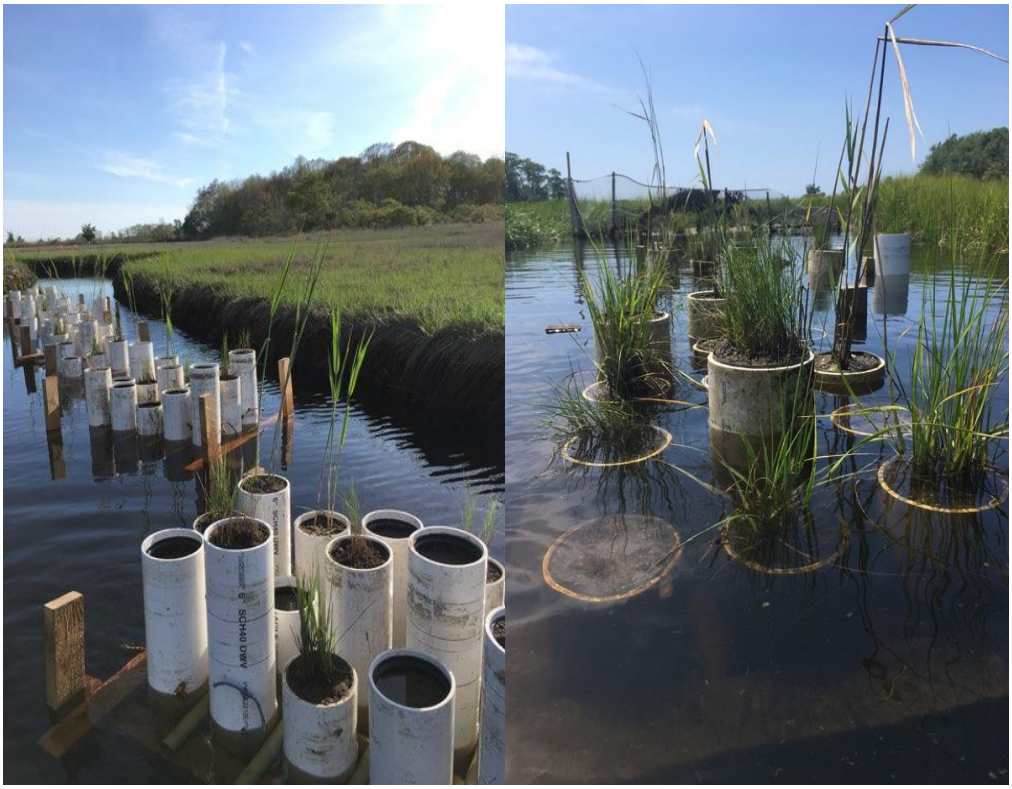

Figure 1. Photos of the marsh organ within Impoundment 3 in Barn Island Wildlife Management Area, Stonington, CT, USA. (A) Organs (five) randomly positioned in the tidal creek during a low tide event. Each organ contains five vegetation treatments x three SLR treatments. (B) A close up image of the marsh organ during a high tide event. Tidal heights varied throughout the experiment, therefore some mesocosms such as the *Spartina patens* in the center of the second row, may not have been entirely flooded each high tide.

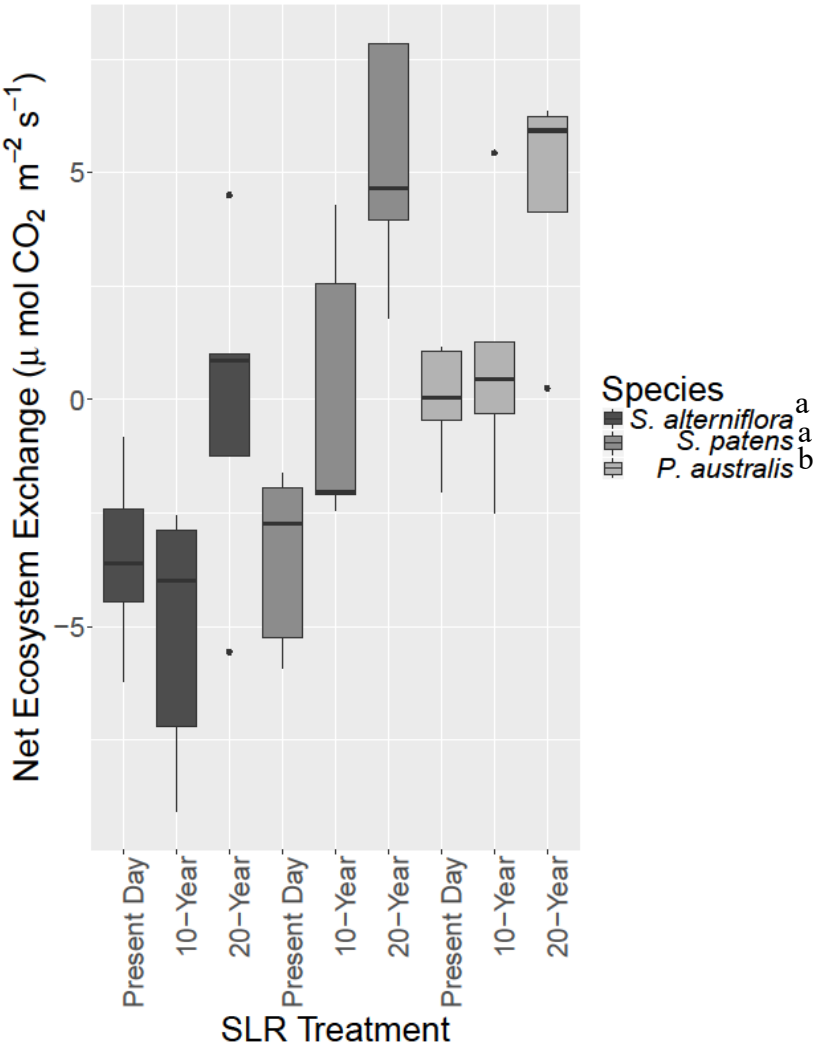

Figure 2. Boxplot of net ecosystem exchange (NEE) of species responses to SLR treatments. Lower and upper box boundaries represent first and third quartiles respectively, line inside box median, lower and upper error lines 1.5*interquartile range, circles data falling outside the range of the error lines. Negative values correspond to carbon uptake, whereas positive values indicate carbon emission. Letters represent significant differences among species. SLR treatments differed with greater emissions as SLR increases, 20-Year SLR treatments had significantly greater emissions than both Present Day and 10-Year SLR. There was no interaction between species and SLR treatments.

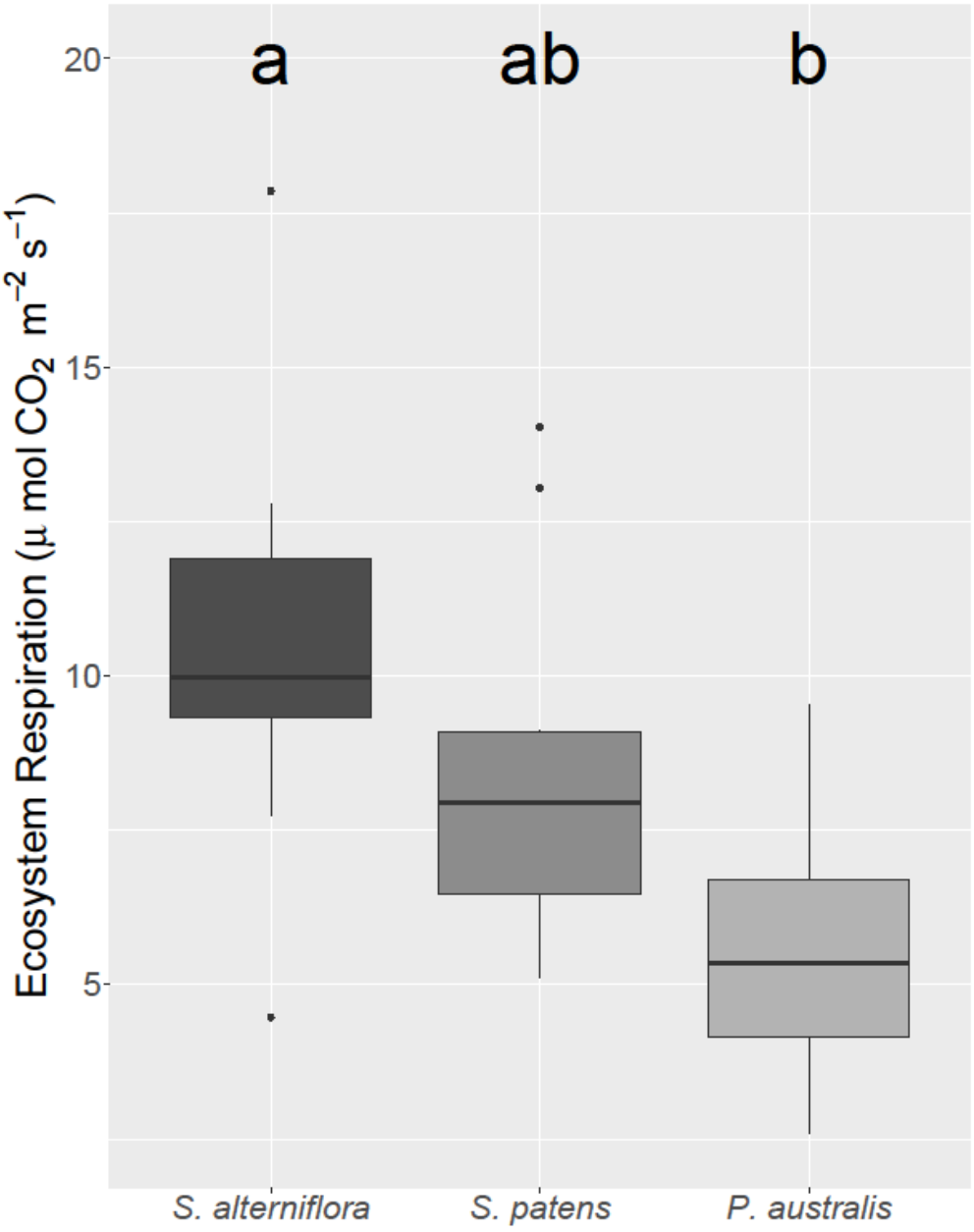

Figure 3. Boxplot of ecosystem respiration (ER). Letters represent ecosystem respiration differences among species. SLR treatments were not significantly different, but ecosystem respiration differed among vegetation zones. Lower and upper box boundaries represent first and third quartiles respectively, line inside box median, lower and upper error lines 1.5*interquartile range, circles data falling outside the range of the error lines.

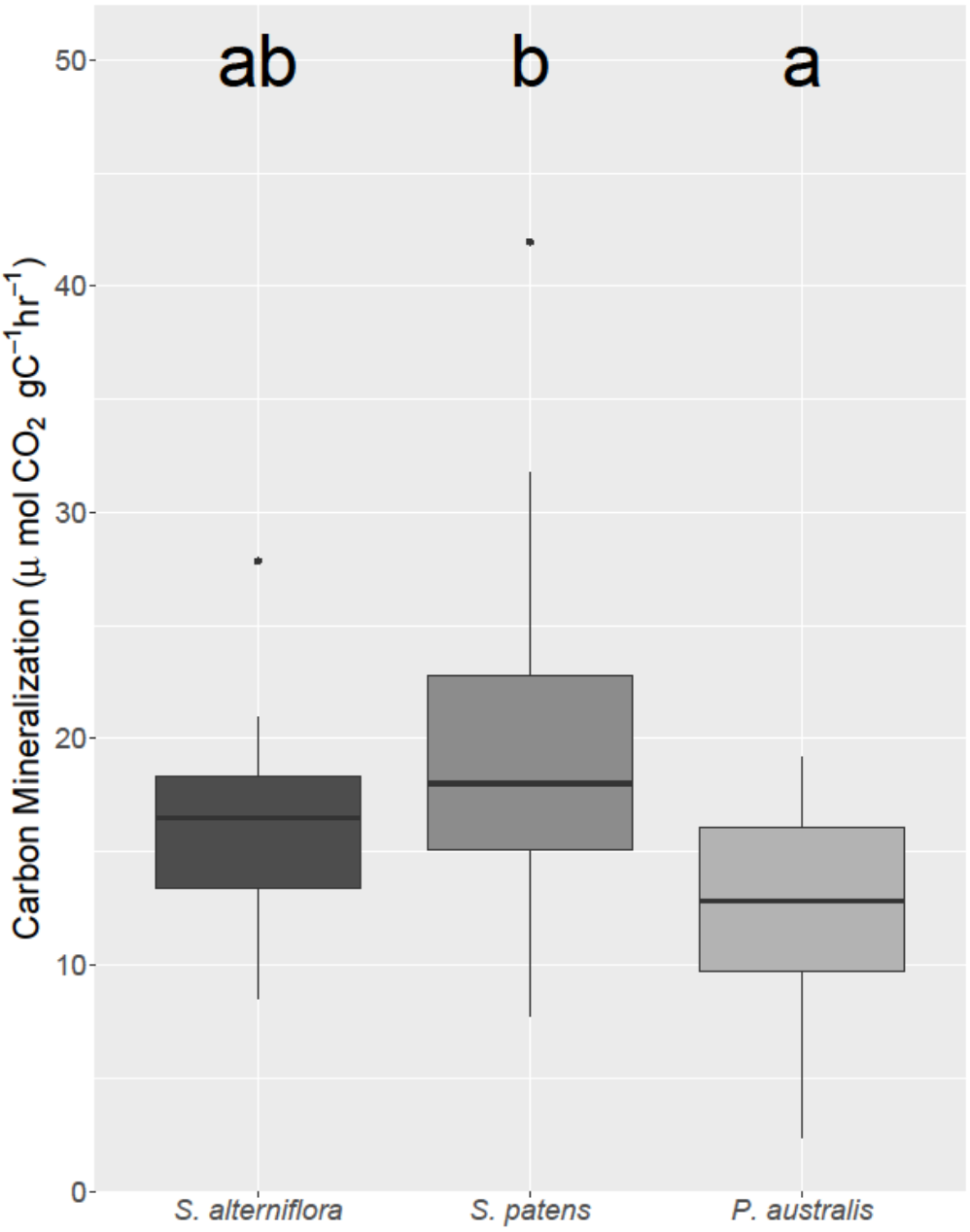

Figure 4. Boxplot of laboratory assay of carbon mineralization. Letters represent significant differences among species. SLR treatments were not significantly different, but carbon mineralization rates differed among vegetation zones. Lower and upper box boundaries represent first and third quartiles respectively, line inside box median, lower and upper error lines 1.5*interquartile range, circles data falling outside the range of the error lines.

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